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SULFHYDRYL PROTECTION OF THE LIVER

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CHICAGO

It was demonstrated in 1939 that preliminary feeding of a high protein diet protects the liver of the experimental animal against chloroform injury.[1] Subsequent studies have shown that preliminary administration of the sulfur-containing amino acid methionine affords similar protection.[2] The experiments to be described were conducted in an attempt to elucidate the mechanism of this protective action of protein and methionine.

METHODS

Mongrel dogs of both sexes, fed a stock diet, were anesthetized with chloroform. The latter was placed in a Mason jar attached to a rubber tube that was connected with a cylindric mask with a rubber diaphragm designed to fit tightly over the snout of the animal. By means of a valve on the screw-cap of the jar, varying admixtures of air and chloroform were afforded the animal. Usually 1.6 cc. of chloroform per kilogram of body weight was placed in the jar and surgical anesthesia induced. The time required for complete evaporation of the chloroform was noted and varied from forty-five to sixty-five minutes. Twenty-four hours later and on each subsequent day for six to eight days, tests of liver function were made with sulfobromophthalein sodium. Five milligrams of the dye per kilogram of body weight was injected, and blood was withdrawn in twenty minutes for colorimetric estimation of the dye retained in the serum. After a rest period of at least two months, the anesthesia was repeated under practically identical circumstances and sulfobromophthalein tests again were carried out daily. The degree of hepatic damage, or the rapidity of the return toward normal, after the first period of anesthesia was compared with that after the second. At the onset of the second period of anesthesia various substances were injected, usually during the first fifteen to twenty minutes, to observe whether or not the subsequent hepatic damage was the same or less than after the first period of anesthesia in that animal.

From the Departments of Surgery and Medicine, University of Chicago.

This work was facilitated by the Charles H. and Mary F. S. Worcester Memorial Fund of the University of Chicago.

1. Goldschmidt, S.; Vars, H. M., and Ravdin, I.: J. Clin. Investigation **18**:277, 1939.

2. Miller, L. L.; Ross, J. F., and Whipple, G. H.: Am. J. M. Sc. **200**:739, 1940.

CONTROL STUDIES

To validate this method of determining whether various agents exercise a rapid protective action on the liver, six control experiments were carried out. In 2 dogs, nothing was injected at the onset of the second period of anesthesia, and the degree of hepatic damage was found to be practically identical with that observed following the first period of anesthesia. In 2 animals 3 Gm. of dextrose per kilogram of body weight was injected as a 10 per cent solution at the onset of the second period of anesthesia; in 2 dogs 3 Gm. of gelatin (which does not contain methionine) as an 8 per cent solution in isotonic solution of sodium chloride was injected at the second period. In each instance the degree of hepatic damage was the same or slightly aggravated as compared with that following the first period of anesthesia.

It has been stated that dogs may become resistant to the effects of chloroform after being anesthetized repeatedly, but under the conditions of these experiments no evidence of increased resistance was noted. Indeed, it was apparent that a given degree of hepatic injury as defined by the sulfobromophthalein test was reproducible or on the second occasion was slightly aggravated.

There is considerable individual variation in the degree of hepatic damage resulting from chloroform anesthesia. Thus the results of sulfobromophthalein tests in one animal are hardly comparable with those in another. However, the results of several tests in the same animal are comparable with each other. Composite curves were drawn of the percentages of the sulfobromophthalein sodium retained in the control experiments and are reproduced in chart 1*A*.

EXPERIMENTS

Experiment 1.—Seven dogs were used. An enzymic digest of casein (amigen) in 10 per cent solution was injected at the onset of the second period of anesthesia. The doses of the dry material per kilogram of body weight were: 1.6 Gm. in 1 animal; 2.3 Gm. in 1 animal; 2.6 Gm. in 1 animal; 4.0 Gm. in 3 animals; 4.6 Gm. in 1 animal. The methionine content in these quantities varied from 48 to 138 mg. (casein contains 3 per cent methionine).

The composite curves shown in chart 1*B* indicate a degree of protection. When the test animals are considered individually, 3 dogs showed no protection, 2 marked protection and 2 moderate protection.

Experiment 2.—Five dogs were used. dl-Methionine ($CH_3SCH_2CH_2CHNH_2COOH$) dissolved and suspended in 50 cc. of saline solution was injected intravenously at the onset of the second period of anesthesia.

The doses varied from 88 to 170 mg. per kilogram. (This is the methionine equivalent of 2.9 to 6.2 Gm. of casein digest.) Moderate to marked protection was observed in each animal. The composite curves are shown in chart 1*C*; methionine afforded appreciable protection.

Experiment 3.—Six dogs were used. Sodium thioglycollate ($NaOOCCH_2SH$) was injected intravenously at the onset of the second period of anesthesia. It was prepared as follows: thioglycollic acid was added to saline solution and the p_H adjusted to 7.4 by addition of powdered sodium carbonate.

The doses varied from 98.5 to 197 mg. of thioglycollate (calculated as thioglycollic acid) per kilogram of body weight. In 1 dog 120 mg. per kilogram of body weight was injected twenty-four hours before the second period of anesthesia, 120 mg. five hours previously, 120 mg. at the onset of anesthesia and 120 mg. at the termination of anesthesia. Ninety-eight and five-tenths milligrams of thioglycollic acid represents the sulfur equivalent of 160 mg. of methionine, and 197 mg., the sulfur equivalent of 320 mg. of methionine.

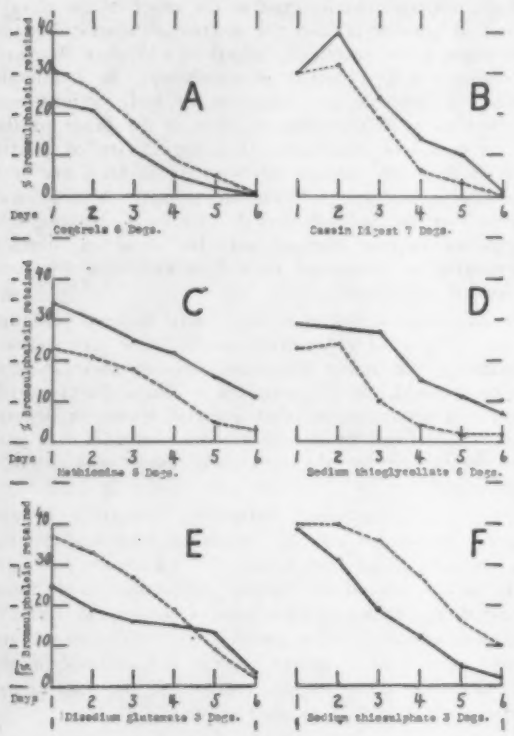


Chart 1.—Composite graphs of the results of sulfobromophthalein tests in five groups of dogs receiving various substances in an attempt to demonstrate which exert definite protective action against chloroform injury of the liver. The solid line represents the percentage of sulfobromophthalein sodium retained in the serum after an initial, or control, period of chloroform anesthesia; the broken line, that after a second period of chloroform anesthesia in the same animals at the outset of which period the protective agent or other substance was injected intravenously. *A* shows greater injury of the liver occurring after the second period of anesthesia when no injection or a control injection was made. *B*, *C* and *D* show less hepatic injury occurring after injection of casein digest, methionine and sodium thioglycollate, respectively. *E* and *F*, control studies, show failure of protective action on the part of disodium glutamate and sodium thiosulfate; indeed, there appears to have been additional hepatic injury, especially after injection of the latter.

Composite curves of the results are shown in chart 1*D*. Moderate to pronounced protection of the liver was observed in each animal of this group. As might be expected, the greatest degree of protection was noted when multiple doses of thioglycollate were injected.

Experiment 4.—Three dogs were used. Sodium glutamate ($NaOOCCH_2CH_2CHNH_2COONa$), prepared by neutralization of glutamic acid with sodium hydroxide, was injected at the onset of the second period of anesthesia. The dose injected in each instance was 320 mg. (glutamic acid) per kilogram. This represents the nitrogen equivalent of 320 mg. of methionine. In each of the 3 dogs the damage of the liver was greater after the second period of anesthesia than after the first period. Thus $—NH_2$ does not afford protection. The composite curves are shown in chart 1*E*.

Experiment 5.—Three dogs were used. Sodium thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$) was injected at the onset of the second period of anesthesia. The doses varied from 132 to 396 mg. per kilogram. These represent sulfur equivalents of 160 to 480 mg. of methionine (the latter representing the equivalent of 5.28 to 15.84 Gm. of casein per kilogram of the dog's weight). In each instance no protection of the liver was observed. The composite curves are shown in chart 1*F*. Indeed, the hepatic damage was greater after the second period of anesthesia.

COMMENT

Control experiments, the details of which are not related here, demonstrated that dilution effects protecting the liver were observed if at least 50 cc. of fluid per kilogram was rapidly injected into the circulation at the onset of anesthesia. In all experiments cited in the foregoing section the total quantity of fluid injected did not exceed 50 cc. except in the animals which received casein digest, and here the quantity was less than 50 cc. per kilogram.

Casein digest afforded irregular protection of the liver, and in some instances no protection was observed. Methionine afforded protection in each of the 5 dogs employed in the experiment. Sodium thioglycollate afforded protection in each of the 6 dogs tested. Sodium thioglycollate and methionine both contain sulfur. In the former it is present as an — SH group. Without presenting a detailed discussion, it may be stated that at least part (if not most) of the sulfur administered to the organism in methionine becomes available as an — SH group. Experiment 4 was carried out to control the possibility that a 5 carbon atom chain amino-acid might afford protection apart from the presence of sulfur, since methionine contains 5 carbon atoms. These experiments did not show protection and thus also served to control the possibility that $— NH_2$ might afford protection. Experiment 5 was carried out to control the

possibility that sulfur not as — SH might afford protection. This experiment also gave a negative result. (The protective effects are shown correlated with structural formulas in chart 2).

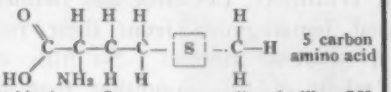
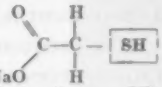
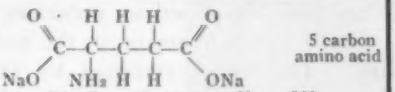
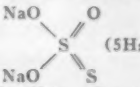
Agent	Liver Protection
Protein Molecules That Contain —SH groups.	Positive (In sufficient quant.)
 5 carbon amino acid Methionine —S— present as "masked" —SH	Positive
 Sodium thioglycollate. —SH present.	Positive
 5 carbon amino acid Disodium glutamate. No —SH	
 Sodium thiosulfate. S not as —SH	Negative Negative

Chart 2.—Structural formulas correlated with protective action against chloroform injury of the liver. There is protection when —SH is present but not when NH_2 is present alone or with sulfur not as —SH (as in sodium thiosulfate).

It was not deemed necessary to control the action of the "labile methyl group" in methionine inasmuch as protection was obtained with sodium thioglycollate, which does not contain this group.

It thus appears that — SH administered to the organism at the onset of chloroform anesthesia affords a degree of rapid protection of the liver against injury by this anesthetic agent. In some individual experiments the rapid protective action of — SH was manifested not as a reduction in the degree of hepatic injury present twenty-four to forty-eight hours after anesthesia (although this was often the case) but as an appreciably more rapid return toward normal hepatic function than was observed after the control period of anesthesia.

Failure to observe better protection in experiment 1 with casein digest may be explained by the insufficient quantity injected (insufficient quantity of — SH).

The data presented suggest that protein and methionine exert protective action against the hepatic injury of chloroform by virtue of their — SH components.

SUMMARY

The experimental evidence presented shows that — SH administered to dogs at the onset of chloroform anesthesia in the form of sodium thioglycollate affords a degree of rapid protective action against hepatic injury. This effect parallels that observed with methionine. The protective action of — SH obtains apart from the concomitant administration of $— NH_2$ (in a 5 carbon atom chain amino acid). Sulfur (other than — SH) as sodium thiosulfate failed to confer protection against hepatic injury.

HEMATURIA DUE TO PAPILLARY HEMANGIOMA OF THE RENAL PELVIS

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Hemangioma of the kidney is a rare disease which has been receiving increasing attention from urologists and pathologists in the last decade. Although Virchow,[1] in 1867, was probably the first to demonstrate this tumor at autopsy, it was not until 1903 that Fenwick [2] first defined its clinical manifestations and demonstrated that the anatomic basis of the obscure hematuria was the presence of dilated veins in the submucosa of the renal pelvis. This lesion he termed "capillary hemangioma." In a review of the literature in 1937 Dalakopoulous [3] listed 41 reported cases. Four years later Riley and Swann [4] could accept only 36 of these as true cases of angioma. Additional reports of single cases have recently appeared, by Farkas,[5] Dean and McCarthy,[6] Riley and Swann,[4] McLean and Mathews [7] and Shaheen, Cassano and Lisa,[8] and a report of 3 cases by Rottino and Mohan.[9]

Additional evidence of the infrequent occurrence of this tumor is supplied by the data of Riley and Swann, who found no case of renal hemangioma in 13,219 consecutive autopsies at Boston City Hospital. Rarely, if ever, is the possibility of renal hemangioma considered in the differential diagnosis of hematuria.

Melicow [10] does not even include this disease in his classification of 199 renal neoplasms from Squiers' Clinic and makes only slight mention of cancerous hemangioblastoma in reference to a case of von Hippel's disease.

Similarly, Whitmore, LeComte and Bennett [11] omitted renal hemangioma from their recent classification of renal tumors. So little cognizance is taken of this condition that they aver that benign tumors of the kidney are of little importance. This statement is hardly borne out in the literature by the frequent description of "emergency operation–necessitating hematuria" caused by this "benign" lesion.

Angioma has been described as occurring in all portions of the parenchyma or in the pelvis of the kidney. The tumor reported by Dean and McCarthy [6] was located in the inferior half of the lower pole. In Gile's [12] case the neoplasm comprised one half of the kidney and resembled a massive sponge, while in the case reported by von Szecsey and Flesch [13] a palpable mass was demonstrated in an infant. Involvement of the pelvis has been confined usually to the calices with particular localization adjacent to the tip of a papilla. The tumors have been described as ranging in size from pinpoint to 6 to 8 cm. in diameter; they are either subepithelial or papillary in character and never involve the capsule (Simpson [14]; Baum [15]; Stieda [16]).

Clinically, the onset of hematuria has occurred before the age of 40 in 80 per cent of the cases. It has been characterized by intermittent or constant bleeding, ranging from an insignificant hemorrhage to one necessitating an emergency operation. No racial or sexual predominance has been noted. A relationship of renal angioma to multiple angiomatosis of the skin has been suggested by Dean and McCarthy.[6] It has been pointed out by Riley and Swann [4] that every

1. Virchow, R., cited by Swan, R. H. J., and Balme, H.: Brit. J. Surg. **23**:282, 1935.
2. Fenwick, H.: Handbook of Clinical Electric-Light Cystoscopy, London, J. & A. Churchill, 1904; cited by Riley and Swann.[4]
3. Dalakopoulos, N.: Ztschr. f. Urol. **31**:458, 1937.
4. Riley, A., and Swann, W. J.: Urol. & Cutan. Rev. **45**:377, 1941.
5. Farkas, K.: Virchows Arch. f. path. Anat. **304**: 473, 1939.
6. Dean, A. L., and McCarthy, W. D.: Tr. A. Am. Genito-Urin. Surgeons **33**:1, 1940.
7. McLean, E. H., and Mathews, T. J.: West. J. Surg. **50**:47, 1942.
8. Shaheen, A. L.; Cassano, C., and Lisa, J. R.: J. Urol. **51**:597, 1944.
9. Rottino, A., and Mohan, H.: J. Urol. **51**:601, 1944.
10. Melicow, M. M.: J. Urol. **51**:333, 1944.
11. Whitmore, E. R.; LeComte, R. M., and Bennett, W. W.: Urol & Cutan. Rev. **48**:157, 1944.
12. Gile, H.: Surg., Gynec. & Obst. **48**:555, 1929.
13. von Szecsey, G., and Flesch, A.: Ztschr. f. Urol. **27**:866, 1933.
14. Simpson, G.: Proc. Roy. Soc. Med. (Urol. Sect.) **20**:728, 1926.
15. Baum, E. W.: Deutsche. Ztschr. f. Chir. **125**: 520, 1913.
16. Stieda, A.: Beitr. z. path. Anat. u. z. allg. Path. **71**:545, 1923.

nephrectomy for this disease has been followed by cure; there has been no operative mortality.

A preoperative diagnosis of renal hemangioma has never been made and only once has been considered. The usual differential diagnostic considerations have included tuberculosis, carcinoma and calculus. Tuberculosis could be ruled out by the absence of acid-fast organisms of calculi could not be supported because of the absence of roentgen shadows, although frequently the lumbar pain of hematuria was described as similar to the renal colic due to stones. Pyelographic studies usually revealed pathologic changes. These were characterized as "filling defects of the pelvis or calices," "distortion of contour" or "blunting of calices" and were

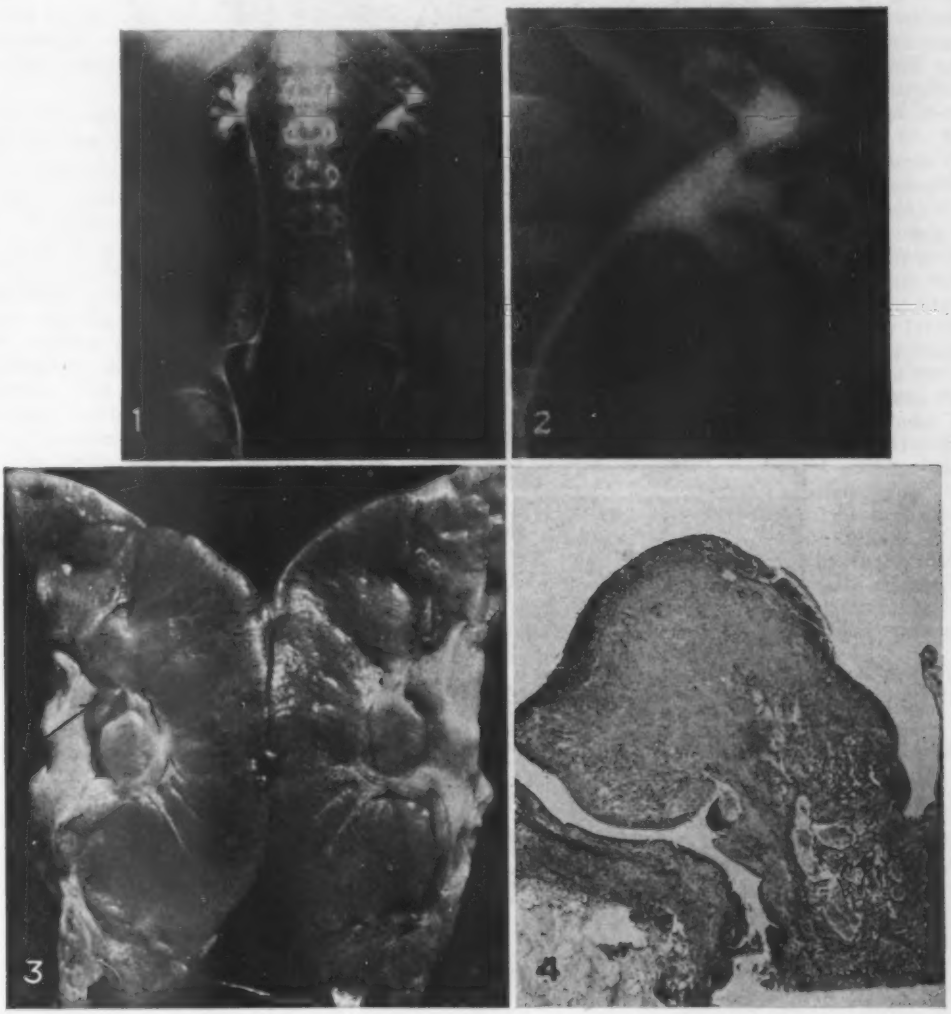

Fig. 1.—Bilateral retrograde pyelogram showing an irregular filling defect in the upper calix of the left kidney, which was considered to be due to either neoplasm or blood clot. The right kidney is aplastic, and the blunted calices and the dilated ureter presumably show the effects of an old infection.

Fig. 2.—Enlargement of the left pyelogram showing the filling defect in the upper calix.

Fig. 3.—Arrow indicates papillary structure in the upper calix. The surface is dark, discolored and rather rough.

Fig. 4.—Photomicrograph showing the papillary structure of the mass and the large, dilated sinusoids, as well as the cavernous arrangement (× 16).

and by the presence of pus in the urine. Carcinoma could be discarded in view of the fact that the majority of patients were below 40 years of age and the other clinical findings of cancer, such as palpable mass, pain, wasting and metastases, were invariably absent. The diagnosis usually interpreted as denoting the presence of tumor or tuberculosis.

The histologic changes have been variable. In several instances only dilated, thin-walled, blood-filled spaces with flat endothelium were seen. In others a more differentiated type of

vessel wall could be determined by the presence of smooth muscle and elastic tissue. In rare instances the wall was so thick as to elicit a diagnosis of arterial hemangioma. The most common finding has been a combination of these types associated with a cavernomatous lesion resembling in all respects similar tumors of the liver and the skin. A variation in terminology was introduced by Jacobs and Rosenberg,[17] who preferred to speak of the morphologic changes as telangiectasis rather than as hemangioma. No useful purpose, however, is served by this differentiation. Usually there has been no indication of growth manifested by active formation of new vessels or the establishment of solid endothelial tumors, although frequently such expanding growth could be determined inferentially by the compression of surrounding interstitial connective tissue. On several occasions the vessels contained thrombi in advanced stages of organization.

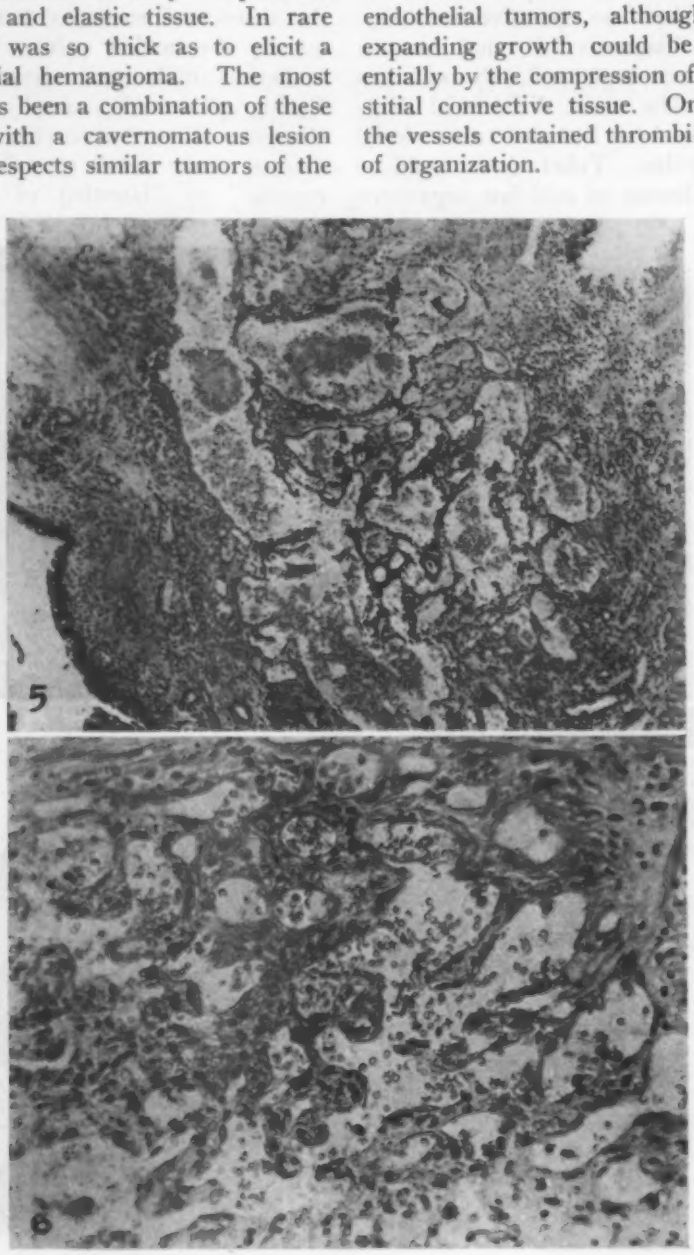

Fig. 5.—Photomicrograph of the apex of the papilla disclosing the markedly dilated sinusoids and the telangiectatic vessels. The chronic inflammatory induration is also evident (× 180).

Fig. 6.—Photomicrograph of the characteristic cavernous arrangement of the vessels, which are lined by a well developed endothelium (× 420).

17. Jacobs, P. A., and Rosenberg, W.: J. Urol. **17**: 337, 1927.

REPORT OF A CASE

A 34 year old white man was admitted to this hospital July 29, 1944 from the Southwest Pacific theater of operations with a transfer diagnosis of "pyelonephritis, acute, suppurative, moderate, of the right kidney." He complained of pain in the left flank and stated that he had had constant hematuria for four months, associated with severe colics on that side. Urinalysis disclosed large numbers of red cells and moderate albuminuria. Blood counts revealed advanced

normocytic anemia. Pyelographic studies showed a constant filling defect in the upper calix of the left kidney (figs. 1 and 2), which was otherwise of normal size and shape.

The patient was treated by heminephrectomy on August 14. The postoperative course was complicated by acute atelectasis of the lower lobe of the right lung, which cleared up in three weeks and for which the patient received a total of 4,200,000 units of penicillin intramuscularly.

The patient continued to show occasional pus and red blood cells in his urine for three months thereafter, but these gradually disappeared and were interpreted as representing subsiding pyelonephritis of the right kidney.

The patient was discharged as cured to a convalescent hospital on Jan. 10, 1945.

Pathologic Examination of the Operative Specimen.—The specimen consisted of one third of the upper pole of the left kidney, weighing 38 Gm. and measuring 6.5 by 3 by 4 cm. The usual perirenal fat was present. The capsule stripped readily, exposing a smooth, somewhat brown, moist surface. The kidney was bisected, exposing the pelvis and the calices. The usual renal structure stood out prominently; the cortical and medullary portions were of the usual width and appearance. No gross abnormalities were noted within the renal parenchyma. Immediately contiguous to a papilla of a posterior-superior calix and apparently arising directly from the pelvic mucosa was a small pea-shaped, pea-sized tumor mass, which was attached to the pelvic lining by a rather broad pedicle (fig. 3). The mass was dark in color and rough on its superior surface, but the underlying surface revealed a somewhat pale, smooth appearance. The mass was in contact with the surrounding pelvic mucosa, which was granular, thickened and of dusky hue. The remaining portions of the pelvic lining revealed no notable alterations, the surface being smooth and pale.

The specimen was prepared for microscopy so as to allow for sections through the mass, the pedicle, the attachment and the surrounding tissue (fig. 4). The tumor was a polypoid mass, the superficial epithelium of which was largely absent, being replaced by a leukocytic-hemorrhagic exudate in a moderately advanced stage of organization. The inferior aspect of the mass and pedicle showed transitional epithelium which deflected into and was continuous with the pelvic epithelium. The stroma of the polyp was usually dense and fibrous and in places was collagenous and avascular although infiltrated by large numbers of round and plasma cells and polymorphonuclear leukocytes, including eosinophils. The greater portion of the mass, however, consisted of widely ectatic vessels, resembling cavernoma. Many of these vessels were thick walled and fibrous and revealed a rather usual-appearing endothelial lining (fig. 5). Portions of septums were seen which extended into the lumens as in a cavernomatous mass. Other areas were composed of a net of engorged, dilated, thin-walled capillaries. Enlarged, ectatic vessels were present also in the tip of the papilla. These were multiloculated spaces of irregular size and shape, showing considerable papillary infolding of the endothelial lining and surrounded by dense masses of connective tissue (fig. 6). Some of the vessels resembled varicosities. Apart from the cavernomatous areas, many isolated young capillaries were seen which were proliferating into granulation tissue. The epithelium of the surrounding pelvic lining as well as of the pedicle was thickened and hyperplastic. The subcutaneous tissues throughout were densely infiltrated by a large number of plasma cells particularly, which were very prominent, as well as by many lymphocytes and eosinophils. Edema and fibrosis were prominent, and there was also considerable engorgement of the smaller capillaries. The inflammation extended into the peripelvic fat where involvement of the interlobular septums and perivascular regions was exhibited. Hyperplasia of lymph follicles was present, several nodules being seen in the subendothelial region of the pelvic lining. Portions of renal parenchyma distant from the mass showed a slight degree of fibrosis and some cellular infiltration of a chronic nature, accompanied by engorgement of a moderate degree. However, no specific degenerative or inflammatory changes of the tubular structure of the papillae could be seen. The remaining portions of the kidney were not altered in appearance.

CONCLUSION

The increasing frequency of reports of cases of papillary hemangioma of the renal pelvis makes it advisable to consider this lesion in every case of hematuria in which the patient is below the age of 40.

CANCEROUS SYNOVIAL TUMORS

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Careful microscopic study has led to the identification of a special group of mesenchymal tumors which originate in the synovial lining of joints, in bursae and in tendon sheaths and which are nearly always cancerous. Though several reports with adequate histologic description of the type of neoplasm represented in this group have appeared during the last ten years,[1] the number of authentic cases is still small. This can only partially be explained by the fact that the synovial tumor is not always recognized as such. The longest individual series has been reported by De Santo, Tennant and Rosahn [1g]; it contains 16 cases, which were collected during a period of at least seven years from hospitals of several large cities. Fisher,[1i] giving an extensive review of the literature, lists 45 cases, including his own 2 cases; with the cases of De Santo and with that of Gross and Cameron [1h] the total number amounts to only 62 cases. I feel therefore justified in reporting 3 additional cases, in 2 of which a very complex structure of the tumor was shown.

REPORT OF CASES

CASE 1.—A Negro man, a native of St. Martin, Netherlands West Indies, and 58 years old, was admitted to the hospital with the diagnosis of cancerous tumor of the knee. The region of the left knee was occupied by a large tumor, measuring approximately 15 by 13 by 10 cm., which had completely obscured the anatomic landmarks. It had been present for a long time, but it was impossible to determine the duration of the symptoms. The consistence of the tumor varied but was never firm. The joint was kept in semiflexion; movement of the joint was impossible. Roentgen examination showed far advanced destruction of the patella, of the femoral condyles and of the head of the tibia. Inguinal or pulmonary metastases could not be demonstrated. High amputation was performed (Dr. M. J. Hugenholtz), and after an uneventful postoperative course the patient returned to his native island. He died eight months after the operation; the exact cause of death could not be determined.

The tumor tissue was whitish gray and of varying consistence. Several areas of softening were seen. The destruction of bone found at dissection confirmed the roentgen diagnosis. There was also extensive infiltration of the soft tissues.

Microscopic Examination.—Numerous blocks of tissue were fixed in Heidenhain's "Susa" mixture.[2] The sections were stained with azocarmine–aniline blue, Masson's tetrachrome, orange–eosin–toluidine blue and orcein and by Gömöri's method for the impregnation of reticulum fibers.

In the tumor three cell types could be distinguished, which by their mutual arrangements and by the varying admixture of connective tissue fibers produced a peculiar pattern. The first type was represented by large cells with light-staining, oval, sometimes indented or more elongated nuclei, which contained one, sometimes two, conspicuous nucleoli. In some cells each of the nucleoli was surrounded by a clear halo. The protoplasm also stained lightly. The cells were generally arranged in solid sheets; the cell borders were not distinct. Sometimes the protoplasm was drawn out in thin processes, anastomosing with those of neighboring cells. Here and there the cells were arranged concentrically as squamous epithelium.

The cells representing the second type contained somewhat smaller, darker-staining nuclei of a more irregular form. Though every nucleus contained at least one distinct nucleolus, this was not so conspicuous by reason of the greater chromatin content of the nucleus. The protoplasm also stained darker than that of the cells of the first type; it often contained a homogeneous-appearing area adjacent to the nucleus which stained rose with the orange–eosin–toluidine blue stain and pale blue with the azan method. Individual elements could be more easily distinguished than in the first cell type.

A third cell type was that of slender spindle cells, arranged in bundles.

In all three types mitoses occurred, though in moderate numbers.

In many parts of the tumor the pale-staining cells were arranged in oval or rounded groups or sheets, which were fringed by a more or less broad layer of dark-staining cells of the second type. Especially when the bundles of spindle cells observed between these more solid cell accumulations were edematous and stained lightly, the low power view of the tumor presented a certain resemblance to lymph follicles with light staining germinal centers and rims of darker-staining

1. (*a*) Bonne, C., and Collet, A. M.: Geneesk. tijdschr. v. Nederl.-Indië **75**:1384, 1935. (*b*) Zwahlen, P.: Bull. Assoc. franç. p. l'étude du cancer **24**:682, 1935. (*c*) Knox, L. C.: Am. J. Cancer **28**:461, 1936. (*d*) Black, W. C.: ibid. **28**:481, 1936; **39**:199, 1940. (*e*) Berger, L.: ibid. **34**:501, 1938. (*f*) Albot, G.; Thiebaut, F.; Banzet, P., and Hervy, J.: Bull. Assoc. franç. p. l'étude du cancer **28**:589, 1939. (*g*) De Santo, D. A.; Tennant, R., and Rosahn, P. D.: Surg., Gynec. & Obst. **72**:951, 1941. (*h*) Gross, P., and Cameron, D. W.: Arch. Path. **33**:687, 1942. (*i*) Fisher, H. R.: Am. J. Path. **18**:529, 1942.

2. Gatenby, J. B.; Painter, T. S., and others: The Microtomist's Vade Mecum (Bolles Lee), ed. 10, Philadelphia, P. Blakiston's Son & Co., 1937, p. 50.

lymphocytes. Another peculiar aspect was produced when irregular bands of dark-staining cells radiated from a rounded group of cells of the first type, like spokes from a central hub. In still other places the rims of darker-staining cells surrounding the different groups of cells of the first type were almost completely contiguous, and the cells last mentioned were visible as pale-staining areas in a dark-staining background.

The histologic picture was further complicated by the distribution and the arrangement of the connective tissue fibers. When the azan stain was used, some groups of the pale-staining cells were completely devoid of fibers, whereas others, though rarely, showed a network of thin, anastomosing fibers, which in all probability were intraprotoplasmatic. Many cell groups, however, showed a small number of thick hyaline fibers, which were either irregularly distributed or appeared to radiate from the center of the group of cells, leaving areas without fibers between them. Between the dark-staining cells there were thin, not very distinct fibers. Many times the light-staining cells were separated from the surrounding rim of dark-staining cells by a more or less thick hyaline membrane resembling the basement membrane of epithelium and thus strengthening the resemblance of the pale-staining cells to that tissue. With Gömöri's reticulum impregnation the thick hyaline fibers just described and also the membrane just mentioned were argyrophil; apart from these, hardly any reticulum fibers could be found inside the sheets of pale-staining cells. In contrast to this a rich reticulum was demonstrated by Gömöri's method between the dark-staining cells and the spindle cells; here nearly every cell was surrounded by reticulum fibers. There was often continuity between the coarse fibers, the

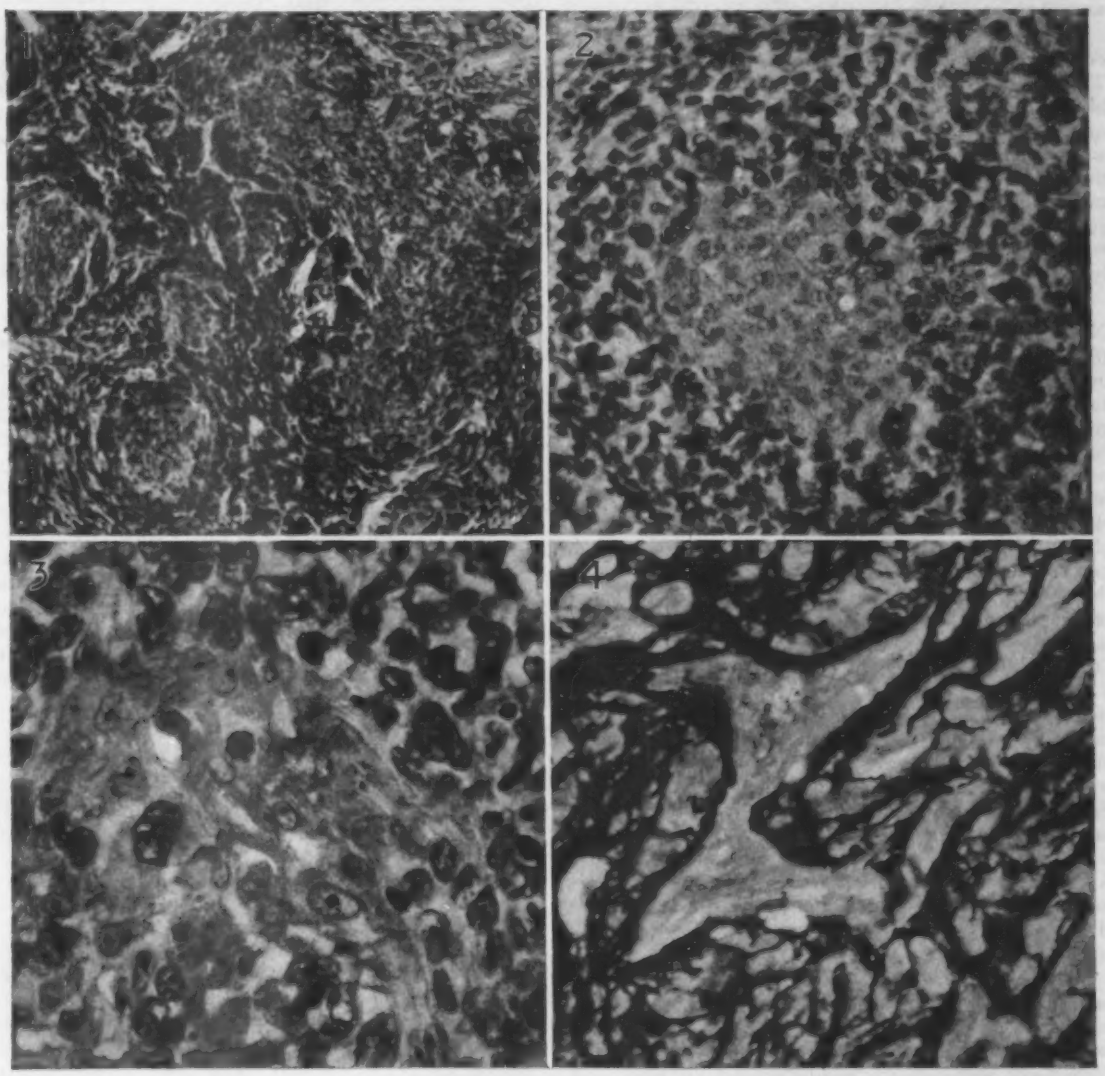

Fig. 1 (case 1).—Rounded and elongated groups of pale-staining cells surrounded by darker-staining elements; × 38.

Fig. 2 (case 1).—Structure somewhat resembling the germinal center of a lymph follicle; × 191.5.

Fig. 3 (case 1).—Pale-staining, epithelium-like cells surrounded by dark-staining elements; × 575.

Fig. 4 (case 1).—Bands of pale-staining cells without reticulum, surrounded by a basement-membrane-like structure and dark-staining cells with reticulum; Gömöri's method; × 240.

basement-membrane-like structures and the reticulum between the dark-staining cells. In other parts of the tumor the arrangement of the cells was different. The pale cells formed narrow, often anastomosing bands, which were again surrounded by the dark-staining cells and spindle cells. The silver impregnation revealed here the same differences in reticulum content: little or no reticulum between the pale cells and a rich reticulum between the dark-staining cells. Structures resembling the basement membranes could be demonstrated here only with the silver impregnation, not with the azan stain, and there were none of the coarse hyaline fibers.

In several places there were small cysts, containing erythrocytes, leukocytes and vacuolated phagocytes. These cysts were lined by large cells somewhat resembling swollen mesothelial cells of the peritoneum. They often contained rounded acidophil bodies, the nature of which could not be exactly determined. Occasionally they showed mitoses. Silver impregnation showed them to be lying sometimes on a kind of basement membrane, just as endothelium does, but in other places to be surrounded by reticulum fibers. Peripherally to these cells hemosiderin-containing phagocytes were seen.

Case 2.—A Negro woman, a native of Curaçao, Netherlands West Indies, 21 years old, was admitted to the service of Dr. F. J. van de Stadt with the following history: During eight months she had been treated for rheuma and syphilis of the left knee joint; after rest in bed her condition improved a little. She then consulted another physician, who made a diagnosis of tuberculosis and applied a plaster dressing. This dressing was removed after nine months, and the patient started walking again. She was seen by still another physician, who advised her to consult a surgeon. Roentgen examination revealed general atrophy of the femur, the tibia, the fibula and the patella. The medial condyle of the femur showed spotty decalcification which extended into the lateral condyle and into the diaphysis. On the medial side and in front of the joint there was a too distinct soft tissue shadow, which also filled the suprapatellar bursa. A biopsy specimen of the joint capsule was taken, and a diagnosis of cancerous, probably synovial, tumor was made. Clinically there were no metastases, and high amputation was performed. Sixteen months later the patient returned with a large tumor of the left groin, which proved to be identical with that of the biopsy and the amputation specimen. A chronic cough developed, and the patient died a few months later, thirty-five months after the appearance of the first symptoms. Autopsy could not be performed.

On dissection of the amputation specimen it was found that the synovial membrane of the knee joint was partially replaced by a layer of reddish gray tumor tissue, which also extended into the suprapatellar synovial pouch and had superficially eroded the bone.

Microscopic Examination.—Blocks of tissue were fixed in Bouin's fluid and in Bouin fluid–mercury bichloride mixture had embedded in Tissuemat.[3] Staining was done with hematoxylin-azophloxine and by the azan method.

The biopsy tissue consisted of more or less loosely arranged cells with relatively little pale-staining protoplasm and large, sometimes oval or bandlike but mostly irregularly indented or multilobed nuclei with varying chromatin content. The more irregular nuclei stained darkest. Nucleoli could be easily found in the oval nuclei but were indistinct or not present in the multilobed ones. Mitotic divisions were numerous. Between the cells slender fibers could be stained by the azan method.

In the amputation-specimen the cells with the indented or lobulated, darker-staining nuclei preponderated in many places. There was marked neoplastic infiltration of the bone in the region of the suprapatellar synovial pouch.

In the inguinal metastasis only cells with lobulated or indented nuclei were seen. The protoplasm formed a relatively narrow rim around the nucleus. The nuclei appeared somewhat paler and the nucleoli larger than in the other specimens just described. Especially when the tumor cells were more loosely arranged, it was difficult to distinguish them from rounded histiocytes. Mitoses were numerous. Very thin, branching fibers were found between the tumor cells.

Case 3.—A white woman 37 years old had been complaining for almost two years of pain in the right foot when walking. She had been treated for flatfoot without any improvement. On examination a rounded swelling of the sole of the foot measuring 4 cm. in diameter was seen. Exploration was done (Dr. M. J. Hugenholtz). A tumor measuring 7 by 5 by 4 cm., larger than expected, was removed. A tendon which was attached to the tumor had to be resected. During operation the impression was gained that the tumor was almost completely encapsulated. It was lobulated, moderately firm of consistence and whitish gray on section. The cut surface showed many fine clefts, which became more distinct after fixation. They contained no blood. A thin tendon was found lying in the middle of the tumor tissue. A histologic diagnosis of cancerous synovial tumor was made and high amputation performed. The period between the amputation and this report is too short to allow a conclusion as to the ultimate prognosis. When the foot was dissected after the amputation, viable tumor tissue, showing many mitoses, was still found.

Microscopic Examination.—Blocks of tissue were fixed in Bouin's fluid and in the Bouin fluid–mercury bichloride mixture [4] and embedded in Tissuemat. Staining was done with hematoxylin-azophloxine, by the azan method,[5] with Petersen's [6] stain and with mucicarmine.

The dominant cell type had a plump oval nucleus containing dustlike chromatin granules and one or two small nucleoli. The protoplasm was finely granular and varied in amount; it stained faintly. Cell borders could not always be defined. Mitoses could be easily found. Though the cells and their nuclei were fairly uniform in size and affinity for stains throughout the tumor, their arrangement and especially the quantity and the quality of the connective tissue fibers lying between them caused variation of aspect. Sometimes the cells were arranged in rounded groups which were encompassed by edematous connective tissue containing small spindle cells; in other places they formed large sheets, often delimited by dense connective tissue resembling rests of fascia or tendon. Also, small isolated groups or nests of tumor cells were found lying inside such

3. Tissuemat is an embedding compound produced by Fisher Scientific Co., Pittsburgh.

4. Hartz, P. H.; van der Sar, A., and van Meeteren, A.: Am. J. Path. **17**:563, 1941.

5. See Heidenhain's aniline blue stain (Mallory, F. B.: Pathological Technique, Philadelphia, W. B. Saunders Company, 1938, p. 154).

6. Peterson, H.: Ztschr. f. wissensch. Mikr. **41**:363, 1924.

dense connective tissue, whereas in other places they appeared to form tumor alveoli, separated by thin septums of connective tissue. Inside such tumor alveoli and also in some of the rounded cell groups there were no fibers staining with aniline blue, so that the cells with their indistinct cell borders resembled epithelium.

When connective tissue fibers were found between the cells, they varied from thin, branching, pale blue fibers to thick, hyaline, dark-staining but still branching ones. In many places the cells, still retaining their characteristic shape and size, were lying as isolated elements or in small groups in dense connective tissue, so that

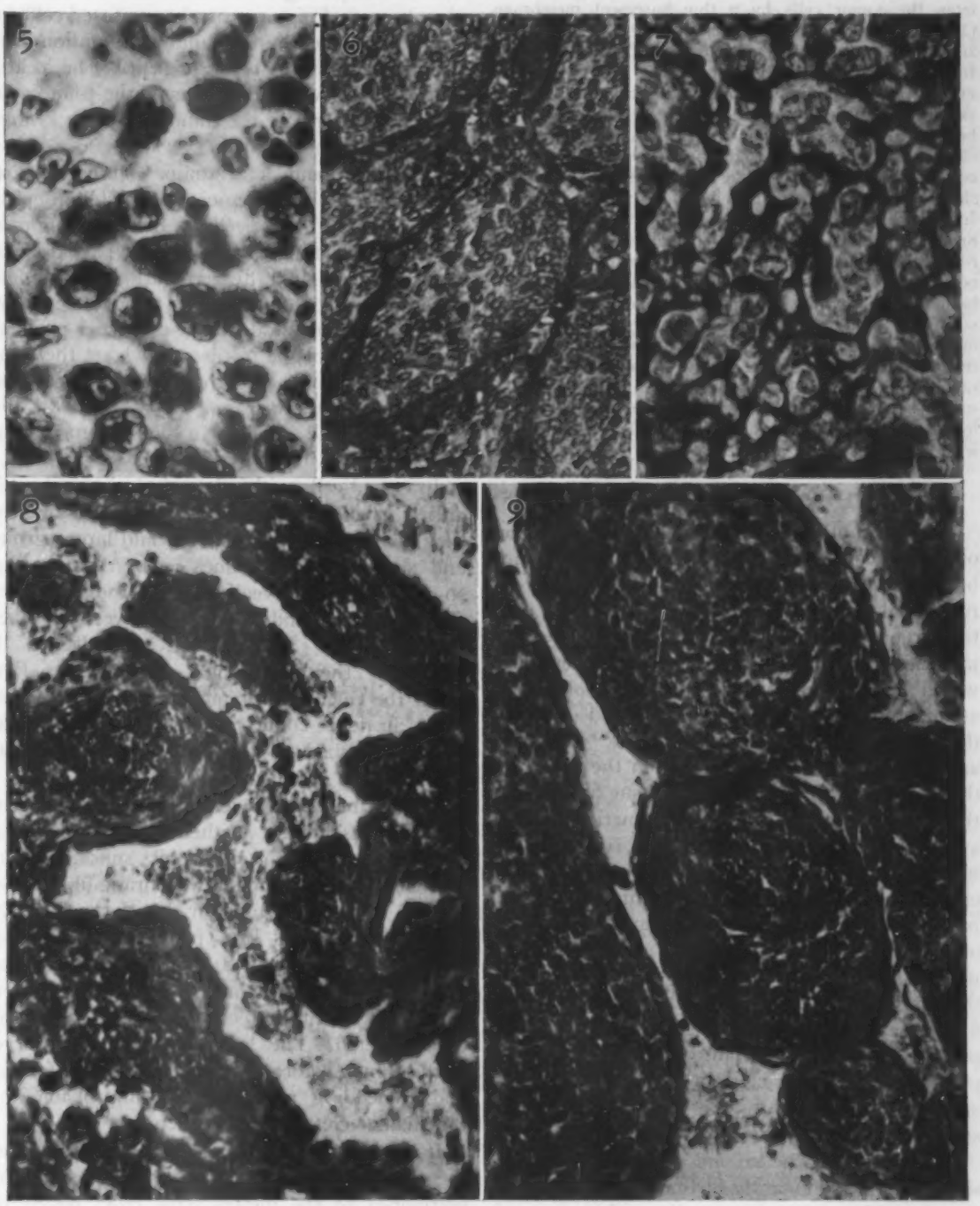

Fig. 5 (case 2).—Tumor cells with indented and lobulated nuclei; × 862.

Fig. 6 (case 3).—Tumor cells resembling epithelium in alveolar arrangement; × 240.

Fig. 7 (case 3).—Tumor cells with thick, hyaline, branching fibers; Petersen's stain; × 575.

Fig. 8 (case 3).—Glandlike spaces; × 240.

Fig. 9 (case 3).—Villus-like structures; × 240.

all transitions from compact cell groups without fibers to dense connective tissue containing isolated cells were present.

There were smaller and larger clefts, often containing a few erythrocytes, in the sheets and groups of tumor cells. Sometimes they could be recognized as capillaries, the flattened endothelial cells being separated from the tumor cells by a thin basement membrane. Other clefts, however, were directly lined by tumor cells and must be considered as the synovial spaces of King. Into the macroscopically recognizable clefts projected villus-like accumulations of tumor cells, containing varying quantities of connective tissue and sometimes, but not always, covered by flattened cells. Other large clefts were directly lined by tumor cells, without the occurrence of the villus-like structures. These large clefts were almost completely free from blood cells. In several places there were small, irregular cavities, lined by flattened or cuboidal, sometimes stratified cells, resembling glandular epithelium. In many places, but not always, there was a thin but distinct basement membrane. The cavities contained an amorphous magma, which in some places gave a positive reaction with mucicarmine. Here and there continuity between the cells lining the glandlike cavities and the ordinary tumor cells was noted. Calcospherites were present in small groups in several places. The small spindle cells found between the groups of tumor cells were identical with those described in case 1.

COMMENT

Various authors (Berger [1e]; De Santo and others [1g]) consider the cells of the synovial membrane as elements capable of assuming different functional and histologic appearances, which tend to be reproduced in synovial tumors. They may present characteristics of so-called border cells, which line the synovial membrane, though *not* as an uninterrupted membrane, a kind of mesothelium, as some authors (e. g., Clark [7]) assume, and are sometimes separated from the cavity of the joint or bursa by connective tissue fibers. On the synovial villi and in inflammation of the synovial membrane the cells may resemble the mesothelium of serous cavities and even true epithelium. These characteristics may be present in tumors having their origin in the synovial membrane and accordingly one must expect tumors exhibiting either histiocytic features or the characteristics of border cells or both. As in many mesenchymal tumors, spindle cells may be present, and, as De Santo and co-workers [1g] pointed out, the characteristics of fibrosarcoma or spindle cell sarcoma may mask true synovial sarcoma.

The tumor in the second case must be considered as predominantly histiocytic. Though in the first biopsy specimen cells with pale oval nuclei were still present, only cells resembling rounded histiocytes were found in the metastasis. They showed a certain resemblance to the cells of the clasmatocytic lymphoma (Gall and Mallory [8]), though in the present case the nuclei were much more irregular and indented. The tumor thus represents the histiocytic type with a unilateral morphologic differentiation of the cells; the absence of pigment or phagocytosed cell rests can be explained by the rapid proliferation of the cells, many mitoses being present. The location of the tumor, which grew inside the joint cavity, makes the origin of the tumor from the synovial membrane almost certain.

In cases 1 and 3 the tumor cells were partly differentiated into elements resembling epithelium. In case 1 these cells by their staining properties, their arrangement and their partial lack of fiber production differed markedly from the other cell types found in this tumor; the mutual arrangement of the different cell types produced a striking histologic picture, which set the tumor apart from spindle cell sarcoma and fibrosarcoma. In one block there was a cavity, partially filled with blood and lined by a synovial-membrane-like border as in Berger's figure 18.

In case 3 cells with relatively large oval nuclei and a small amount of protoplasm dominated; they were arranged in smaller and larger groups and sheets and sometimes in nests resembling so-called tumor alveoli. When the cells were closely packed and when there were no fibers between them, as occurred especially in the "alveoli," they resembled epithelium. Even when the cells were found lying as isolated elements in dense connective tissue, they still could be distinguished from ordinary spindle cells and fibrocytes.

Furthermore, there were glandlike spaces, lined by cells resembling epithelium and sometimes containing a mucicarminophil substance, and also clefts with a synovial-membrane-like border, formed by tumor cells or by flattened endothelium-like cells. Into many of these clefts neoplastic villi projected as in the case of Gross and Cameron [1h] and Black.[1d] Intermediate forms between the different cell types could be easily demonstrated. The large clefts were observed in the tumor before fixation, and careful fixation and embedding made it quite improbable that the smaller clefts were artefacts.

It must be assumed that in this case as in case 1 the tumor cells retained the developmental potentialities of the mesenchyme from which the synovial membrane originates during embryonal development. From this point of view it is interesting to compare the pseudoglandular spaces

7. Clark, W. E. L.: The Tissues of the Body: An Introduction to the Study of Anatomy, New York, Oxford University Press, 1939, p. 142.

8. Gall, E. A., and Mallory, T. B.: Am. J. Path. **18**:381, 1942.

found in case 3 with the mucous cysts of the meniscuses of the knee joint, which are also lined by cells resembling epithelium and contain a mucicarminophil substance. The cells lining these cysts originate from the same mesenchymal elements as the synovial membrane.

All three tumors must be considered cancerous. In case 2 death was probably caused by pulmonary metastases after large inguinal metastases had already been formed. In case 1 the cause of death could not be ascertained, but the large scale destruction of bone and the infiltration of the soft tissues constitute sufficient proof of the presence of cancer. In case 3 the neoplasm infiltrated the soft tissues of the foot and after a seemingly complete extirpation of it, viable tumor tissue, showing numerous mitoses, was found in the amputation specimen. Nor can the cancerous nature of the tumors be doubted histologically, although in cases 1 and 3 an organoid structure was presented.

In case 1 it was probably procrastination of the patient which allowed the tumor to reach its great size; in cases 2 and 3 no tumor was suspected by the physicians who first attended the patient, and a final diagnosis was reached only after biopsy or local excision and microscopic examination. A study of the literature reveals that this is more the rule than the exception. The symptoms caused by the synovial tumors are not characteristic, and the tumors are sufficiently rare for the physician in general practice not to be conversant with them. In typical cases of cancerous synovial tumors the histologic diagnosis is easy. As, however, large parts of the tumor may have an uncharacteristic appearance, large and multiple blocks must be examined (Berger [10]; De Santo and co-workers [15]), and the use of special staining methods is indicated.

SUMMARY

Three cases of a cancerous synovial tumor are presented. In 2 cases the tumor originated in the knee joint and in 1 case in the foot, probably from a tendon sheath. In 1 case the tumor cells were unilaterally differentiated and resembled histiocytes, whereas in the other 2 cases the tumor showed a complex organoid structure, with cells resembling epithelium. In all 3 cases the patient was treated by amputation; in cases 2 and 3 the cancerous nature of the disease was recognized only by microscopic examination.

DIFFUSE HEPATIC NECROSIS CAUSED BY SULFADIAZINE

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Shortly after the sulfonamide compounds were first introduced to the medical profession, there began to accumulate in the literature an increasing number of reports of various untoward reactions, which served as a reminder that these drugs were not altogether innocuous and that they should not be used promiscuously. Some of the reactions were serious, while others were not so serious. Articles [1] on the undesirable effects list the following signs and symptoms as the most commonly encountered: nausea and vomiting, vertigo, headache, cyanosis, "drug fever," cutaneous rashes, renal calculi and renal failure, jaundice and hepatitis, hemolytic anemia, leukopenia, granulocytopenia, conjunctivitis and peripheral neuritis. Because these side effects were relatively numerous with the sulfonamide compounds first put on the market, a series of compounds was developed with the aim of obtaining a derivative whose toxicity would be minimal and whose therapeutic properties would be maximal. Such a compound was found in sulfadiazine, and today this is the drug of choice in most cases.

Although sulfadiazine was much less toxic than its predecessors, physicians soon became aware that even this compound was perhaps not quite as harmless as first expected. Thus, in a total of 906 patients treated with it, Finland, Peterson and Goodwin [2] encountered most of the untoward signs and symptoms seen following the administration of the early drugs. The incidence, however, was far less, and they concluded that "toxic effects attributed to sulfadiazine were relatively few and mild." In the same year Bradford and Shaffer [3] recorded the death of a patient with anuria following the use of sulfadiazine and stated that 2 other patients with anuria had recovered after ureteral catheterization. Subsequently minor reactions in large series of patients were reported by Satterthwaite,[4] and Dowling and Lepper,[1d] and severe reactions resulting in death were reported by Jeck and Orkin.[1c]

Whereas clinically jaundice and damage of the liver were noted to occur not infrequently with the sulfonamide compounds used prior to the discovery of sulfadiazine, recovery was the rule, and deaths with postmortem examinations have been few. In the cases in which necropsy showed changes in the liver, focal hepatic necrosis was reported most frequently,[5] while subacute diffuse hepatic necrosis was reported once and acute diffuse hepatic necrosis four times. The latter occurred each time as a result of sulfanilamide therapy. That jaundice is not a common toxic reaction following the use of sulfadiazine is perhaps best exemplified by the fact that in none of the reports on the side effects of this drug referred to is it even mentioned and that, according to the report of Plummer and Wheeler,[1b] it was seen in only 2 of 1,357 persons treated. Thus, because of the infrequency with which icterus has been noted in patients treated with sulfadiazine, the 2 cases of acute diffuse hepatic necrosis resulting from such therapy which we report are of particular interest and serve as a reminder that no sulfonamide drug should be used with complete impunity.

REPORT OF CASES

CASE 1.—A Negro woman 32 years of age was delivered spontaneously and without anesthesia of a normal full term boy on Oct. 29, 1944. Although the membranes and the placenta were thought to have been delivered completely, on November 3 a small piece of necrotic tissue was observed in the lochia, which histologically proved to be placental tissue. The temperature November 3 was 101.3 F. November 7 she had a chill, a rise in temperature to 103 F. and a burning sensation on urination. The breasts were engorged, and the uterus was soft and tender. A diagnosis of septic endometritis was made, and she was given an initial dose of 2 Gm. of sulfadiazine, followed by 1 Gm. every four hours until a total of 9 Gm. had been administered. Each

From the Clinical Laboratories and Department of Pathology, Jefferson Medical College and Hospital.

1. (*a*) Long, P. H.; Haveland, J. W.; Edwards, L. B., and Bliss, E. A.: J. A. M. A. **115**:364, 1940. (*b*) Plummer, N., and Wheeler, C.; Am. J. M. Sc. **207**:175, 1944. (*c*) Jeck, H. S., and Orkin, L.: J. Urol. **49**:290, 1943. (*d*) Dowling, H. F., and Lepper, M. H.: J. A. M. A. **121**:1190, 1943.

2. Finland, M.; Peterson, O. L., and Goodwin, R. A.: Ann. Int. Med. **17**:920, 1942.

3. Bradford, H. A., and Shaffer, J. H.: J. A. M. A. **119**:316, 1942.

4. Satterthwaite, R. W.: J. Urol. **49**:302, 1943.

5. (*a*) Simon, M. A.: Am. J. M. Sc. **205**:439, 1943. (*b*) Simon, M. A., and Kaufman, M.: Canad. M. A. J. **48**:23, 1943.

dose was accompanied by an equal amount of sodium bicarbonate. November 9 the administration of the drug was discontinued because nausea, vomiting, headache and epistaxis developed. The temperature was 99 F. November 10 the temperature was 104 F., the liver was palpable below the costal margin and there was tenderness in the right upper quadrant of the abdomen. November 12 definite icterus of the scleras was noted, and the patient became drowsy, confused and uncooperative. She continued in this state until her death, November 14.

October 30 the Wassermann and Kahn tests of the blood were negative; November 7 culture of the lochia disclosed no organisms; November 6 and 7 blood cultures were negative. November 11 the blood urea nitrogen amounted to 31.6 mg., and creatinine to 1.5 mg., per hundred cubic centimeters, the direct van den Bergh test was positive, and bilirubin amounted to 13.1 mg. per hundred cubic centimeters. November 12 the blood sulfadiazine level was 7.9 mg. per hundred cubic centimeters. November 13 a bromsulphalein test showed 50 per cent of the dye retained. November 7 to 11 the intake of fluids varied from 1,500 to 3,500 cc. per twenty-four hours and the urinary output from 500 to 2,200 cc. per twenty-four hours; urinalysis before delivery was normal; November 11 the urine disclosed albumin, occasional erythrocytes, many pus cells and granular casts. Several blood counts after delivery showed from 2,775,000 to 3,750,000 erythrocytes per cubic millimeter of blood, and the hemoglobin content was from 65 to 78 per cent. The blood pressure was normal throughout.

At necropsy, twenty-three hours after death, the scleras, the mucous membranes and all the tissues and organs were icteric. The liver weighed 880 Gm. and was considerably reduced in size. The capsule was wrinkled, and the entire organ was soft and flabby. The parenchyma throughout possessed a greenish yellow tint and consisted of irregular mottled areas of light yellowish brown tissue gradually merging with intensely congested tissue or surrounded by fine streaks of frank hemorrhage. The gallbladder and the bile ducts were normal. The left kidney weighed 220 Gm. and the right one 150 Gm. The capsules were not adherent. Cut surfaces showed swollen cortices with everted edges and sharp demarcations. The calices, pelves and ureters were normal. The bladder was distended with dark urine. Nowhere in the urinary tract was a stone or gravel seen. The uterus was larger than normal, but the muscle was well contracted. In the fundus the endometrial surface was covered with a thin patch of greenish yellow tissue, 2 by 4 cm. The mucosa of the small intestine was irregularly congested. The lungs showed some edema. The convolutions of the cerebral hemispheres were slightly flattened. Macroscopically there were no other contributory abnormalities.

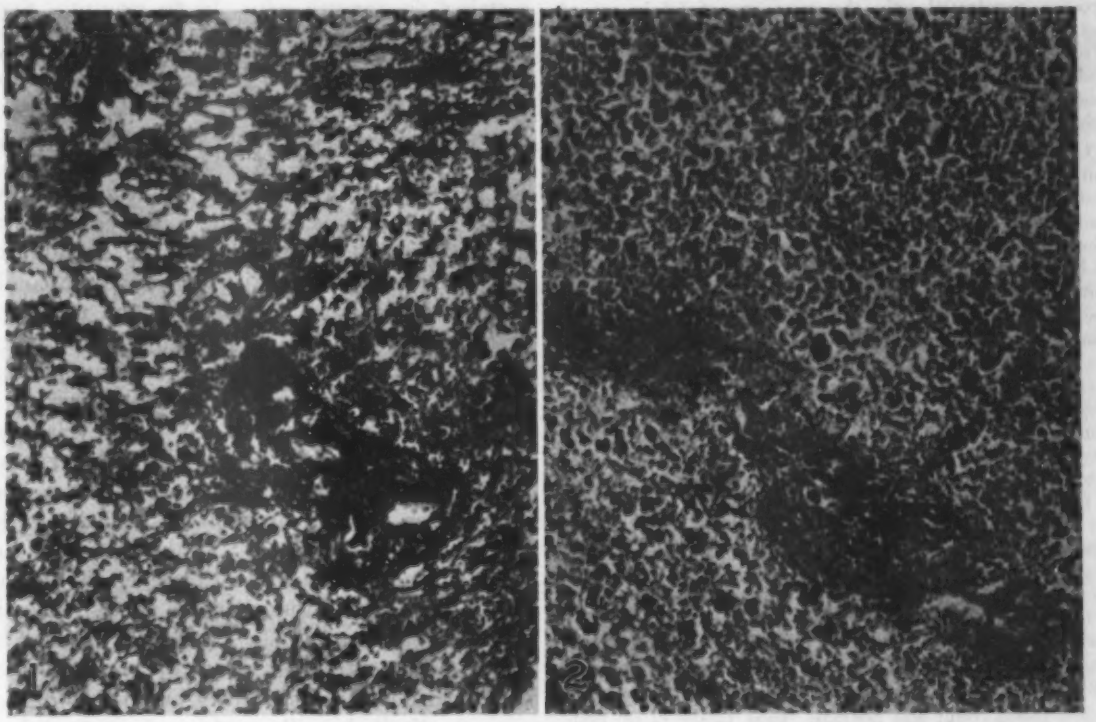

Fig. 1 (case 1).—Photomicrograph of a section of the liver showing a relatively intact portal radicle containing a few cells of inflammatory origin and complete destruction of the liver cells. Hematoxylin and eosin; × 75.

Fig. 2 (case 2).—Photomicrograph of a section of the liver showing a few cells of inflammatory origin in the portal radicles and diffuse necrosis of the liver cells. Hematoxylin and eosin; × 75.

Many sections from various portions of the liver were examined. In all of them, the normal architecture was completely destroyed, and the only recognizable structures were distorted portal radicles (fig. 1). There was questionable early proliferation of the bile ducts, surrounding some of which were collections of plasma cells, lymphocytes and occasional neutrophils. The hepatic cords and cells were completely destroyed. Sometimes they were replaced with foci of complete necrosis, represented by dense collections of pink-staining granular material, but usually the granules were loose, and dispersed between them were shadows of former hepatic cells and large phagocytes loaded with brown pigment. Erythrocytic extravasation was only occasionally seen.

Sections from various portions of the kidneys showed uniform changes. The glomeruli disclosed congestion and frequently precipitated granular material in Bow-

man's spaces. The epithelium of the proximal convoluted tubules showed severe degeneration and swelling, often to such a degree that the lumens were completely obliterated. Their cytoplasm was granular, and their nuclei were either poorly stained or entirely disintegrated. There was less degeneration of the epithelium of Henle's loops and the distal convoluted tubules, but these cells showed considerable sloughing, so that in the more distal portions of the tubules they formed epithelial casts. Calcium salts were deposited in some of the sloughed cells. The cells of the collecting tubules revealed degeneration, but the lumens were free of casts and crystals. The interstitial connective tissue showed considerable congestion, slight to moderate edema, but no inflammatory foci. Sections through the necrotic portion of the endometrium disclosed a superficial layer of nuclear fragments, neutrophils and debris, followed by a thin zone of plasma cells, lymphocytes and phagocytes containing golden brown pigment. This entire process penetrated only the most superficial layers of the myometrium. Placental elements were not recognized. Sections of the lungs revealed congestion, edema and an early stage of terminal pneumonia. The spleen was normal. Sections of the brain showed areas of subarachnoid hemorrhage of moderate degree.

CASE 2.—A white woman 45 years old was first admitted on April 1, 1944, because of swelling of the abdomen and frequent menstruation. These symptoms had been noted for five months. Studies revealed fibromyomas of the uterus producing some obstruction of both ureters, and so the patient submitted to hysterectomy on April 6. Because some fever developed after the operation, sulfadiazine therapy was started April 22. She received 2 Gm. as an initial dose and 1 Gm. every six hours thereafter until a total of 37 Gm. had been received. Complete recovery ensued, and she was discharged from the hospital on May 5. She was well until June 9, when a chill, fever and pain in the back on the left side developed. Her physician made a diagnosis of pyelitis and over a two day period administered 6 Gm. of sulfadiazine along with sodium bicarbonate. At this time she was again admitted to the hospital, where she received 3 Gm. of sulfadiazine in 1 Gm. doses at six hour intervals. The use of the drug was then discontinued because she had a severe chill, a temperature of 106.2 F. and a generalized macular rash. Within the next three days the fever continued and jaundice appeared; the patient became comatose, had several convulsions and died on June 14.

The blood count was normal. June 12 the blood nonprotein nitrogen was 62.5 mg., creatinine 2.11 mg. and the sulfadiazine level 6.7 mg. per hundred cubic centimeters. June 13 the right ureter was catheterized and yielded 300 cc. of urine. On the same day urinalysis showed albumin and granular casts. A blood culture June 14 was negative.

Necropsy, nine hours after death, disclosed generalized jaundice. The liver weighed 1,450 Gm. It was soft and flabby, and its edges were rounded. Both external and cut surfaces disclosed complete disappearance of the normal lobular markings. They were smooth and dull yellowish brown and in the central portion contained numerous dark red punctate hemorrhages. The gallbladder and the bile ducts were normal. Each kidney weighed 210 Gm. The capsules were slightly adherent, and the underlying surfaces were dull reddish brown. There was no dilatation of the ureters, pelves or calices, nor was the mucosa congested. The urinary bladder and the urethra were normal. Gravel or stones were not seen. The lungs showed congestion and edema. The peritoneal cavity contained 1,000 cc. of straw-colored fluid. There were no other contributory abnormalities.

Many sections examined from various portions of the liver showed the same histologic changes as were noted in the first case. There was severe and complete necrosis of the hepatic cells, leaving only granular debris, ghosts of former cells and phagocytes containing brown pigment (fig. 2). The bile ducts were well stained and prominent, and showed no attempt at proliferation. Some were surrounded by various numbers of lymphocytes, neutrophils and plasma cells. Hemorrhages were prominent in some areas but were scanty or altogether absent in others. Sections of each kidney showed marked degeneration of the epithelium of the proximal convoluted tubules, which had a considerable amount of granular debris in their lumens. There was little or no degeneration of the epithelium of Henle's loops and the distal convoluted tubules, but the lumens of both of these segments contained granular, hyaline or "pigmented" casts. The epithelium of the collecting tubules showed degeneration and sloughing, and the lumens contained desquamated cells. In the interstitial tissue were marked congestion of the capillaries, considerable edema and collections of lymphocytes, plasma cells and a few neutrophils. There were congestion and edema of the lungs and congestion of the spleen.

COMMENT

There can be little doubt that the severe damage of the liver in each of the cases recorded was directly related to the action of sulfadiazine rather than to the coexisting pathologic conditions. While it is true that in the case first described nausea, vomiting, headache, epistaxis and later icterus occurred in the puerperium, pregnancy as a cause of the hepatic necrosis is most unlikely for two reasons: First, eclampsia usually occurs in the later part of pregnancy and not in the puerperium, and when it does occur after delivery its onset is usually early. The signs and symptoms in this case did not make their appearance until eleven days post partum. Second, the changes in the liver in eclampsia are practically always manifested by foci of necrosis, either large or small, located at the periphery of the lobules, and not by diffuse necrosis of all the cells. Although acute diffuse hepatic necrosis is known to follow septicemia, this complication is rare, and in this case there was no evidence of septicemia, for two separate blood cultures showed no bacterial growth. The localized inflammation in the endometrium was not the causative factor, because it was minimal and hardly more than one sees at the site of the attachment of the placenta in any uterus. The onset of symptoms, on the other hand, only two days after treatment with sulfadiazine was started is compatible with toxicity arising as a result of administration of this drug.

The concomitant pathologic condition in the second case was certainly not one which warranted even a suspicion that it was the causative factor in the production of the diffuse necrosis of the liver. Chills, fever and pain developing

two days before the patient's last admission could all be attributed to a temporary block of the left ureter, which subsequently opened spontaneously and drained satisfactorily. On the right side this was not the case, for catheterization twenty-four hours before death yielded 300 cc. of urine. At autopsy, neither ureter nor pelvis was dilated, neither was inflamed and neither disclosed the cause of the ureteral obstruction which was obviously present during life. On the other hand, in this case, as in the previous one, the onset of toxic symptoms, consisting of chills, fever and macular rash, followed later by jaundice, occurred approximately two days after sulfadiazine was first administered, and were therefore again within the time in which toxicity to sulfonamide compounds is often manifest.

That hepatic necrosis can occur in man following use of sulfonamide compounds has been well established, but the mechanism involved is obscure. It is thought that either the drug acts directly on the liver in a chemical manner or the action is secondary to an inherent or acquired sensitivity of the organ for the drug. Whether one or both of these theories are correct cannot yet be answered, but it is possible that focal hepatic necrosis is a manifestation of the former and diffuse hepatic necrosis a manifestation of the latter. This contention has considerable support from experiments on animals in which focal necrosis of the liver has sometimes been observed, but diffuse hepatic necrosis has not been produced as far as we know.[5a] The reason for this is that it is extremely difficult to sensitize animals to the sulfonamide compounds,[6] and therefore the action in all instances is probably a simple chemical one. In man, since both focal and diffuse hepatic necrosis have been observed, it is possible that in the former the action is a simple chemical one, while in the latter it is a result of sensitivity. That the liver may be sensitive to the sulfonamide compounds is entirely possible, for other forms of sensitization, particularly the cutaneous, have been well established. It is, for instance, well known that if an ointment containing a sulfonamide compound is applied topically to the skin and this treatment is followed after a short or a long interval by oral administration of the same or another sulfonamide drug, violent eruptions will appear at the site of cutaneous application.[7] Leftwich [8] has recently published a simple method of testing for cutaneous sensitivity to sulfonamide compounds by injecting intradermally serum from patients receiving these drugs. The analogy between serum sickness and the various undesirable reactions to the sulfonamide compounds is most striking, and on this basis Longcope [6] and Leftwich [8] concluded that the fundamental process in the two are the same. If the premise is correct that acute diffuse hepatic necrosis produced in man by a sulfonamide compound results from sensitivity of this organ to the drug, the explanation for such sensitivity in the 2 cases reported here must be different. As far as we know the patient whose case was described first had never been treated with a sulfonamide compound, and so the sensitivity must have beeen on an inherent or natural basis, while the patient whose case was described second received one course of sulfadiazine therapy a month before a second course was administered. This is ample time for true acquired sensitivity to develop.

Because of the frequency with which renal complications have been noted in patients treated with the sulfonamide compounds, this side effect assumes relatively grave proportions. Pathologically two distinct types of renal changes have been described. The first consists of an actual precipitation of the drug within the collecting tubules, calices, pelvis and lower urinary tract, with minor degenerative alterations in the renal tubules. This is the only lesion that has so far been observed in experimental animals and is ordinarily the type seen in man. The second, more recently described renal changes consist of congestion of the glomeruli, presence of edema fluid in Bowman's spaces, severe degeneration and necrosis of the tubular epithelium, dilatation of the lumens of the tubules, with various types of casts in the more distal portions of the nephron, and marked congestion and edematous involvement of the interstitial connective tissue, with foci of plasma cells, lymphocytes and fewer neutrophils.[9] While we agree that the former lesion can be ascribed to the direct toxic action of the sulfonamide compound on the renal tubules, we do not believe there is sufficient evidence to warrant the assumption that the latter process is also directly related to the drug, for essentially similar renal changes have often been described in the literature, and have been repeatedly seen by us, in a variety of diseases that were not treated with the sulfonamide compounds. Some of these conditions were severe

6. Longcope, W. T.: Medicine **22**:251, 1943.

7. Park, R. G.: Brit. M. J. **2**:69, 1943. Shaffer, B.; Lentz, J. W., and McGuire, J. A.: J. A. M. A. **123**:17, 1943.

8. Leftwich, W. B.: Bull. Johns Hopkins Hosp. **74**: 26, 1944.

9. Murphy, F. D.; Kuzma, J. F.; Polley, T. Z., and Grill, J.: Arch. Int. Med. **73**:433, 1944. Prien, E. L.: New England J. Med. **232**:63, 1945.

infections,[10] reactions to transfusions,[11] diseases of the liver with and without jaundice,[12] operative shock,[10] intestinal obstruction and crushing injuries.[13] Because these diseases can of themselves produce anuria, azotemia and the renal changes described, it is not entirely clear to us, and apparently to other authors,[14] how the damage of the kidney can be ascribed to the sulfonamide drug rather than to the initial disease process. The fact that in healthy animals receiving the drug such severe renal changes have not been observed is further proof that the underlying diseases for which the sulfonamide compounds are administered, or alterations which the drug produces—for example, diffuse hepatic necrosis—are partly, if not entirely, responsible for this complication.

SUMMARY

A review of the literature discloses reports on 4 cases of acute diffuse hepatic necrosis resulting from the use of sulfanilamide. To these are added 2 cases of this condition following the administration of sulfadiazine. One patient had not previously received sulfonamide compounds, whereas the other had been given a course of treatment with sulfadiazine one month before a second course was administered.

It is thought that while focal hepatic necrosis may result from outright chemical action of the sulfonamide compounds on the liver cells, diffuse hepatic necrosis may be the result of inherent or acquired hypersensitivity of the liver for the drug.

Of the two types of renal damage ascribed to the sulfonamide compounds, we believe that the one wherein crystals or concretions are found in and beyond the renal tubules is due to the direct action of the sulfonamide compounds on the kidney. The more severe type, however, showing tubular necrosis, casts and interstital congestion and edema, with foci of inflammatory cells, we believe is due to the accompanying disease process, which may be infection, intestinal obstruction, jaundice or some other condition, and not to direct action of the drug on the renal parenchyma.

The 2 cases reported here emphasize the fact that sulfadiazine should be used cautiously.

10. Jeghers, H., and Bakst, H. J.: Ann. Int. Med. **11**: 1861, 1938.
11. Daniels, W. B.; Leonard, W. B., and Holtzman, S.: J. A. M. A. **116**:1208, 1941.
12. Wilensky, A. O.: Arch. Surg. **38**:625, 1939.
13. Bywaters, E. G. L.: J. A. M. A. **124**:1103, 1944.
14. Spring, M., and Bernstein, G.: Ann. Int. Med. **14**:153, 1940.

CYSTIC AND COMPACT PULMONARY SCLEROSIS IN PROGRESSIVE SCLERODERMA

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CYSTIC SCLEROSIS

In 2 cases of scleroderma peculiar cystic changes were observed in the lungs. I have not been able to find a description of similar observations in the literature at my disposal. There was a striking similarity of the topographic distribution of the cysts in the 2 cases. The zones containing cysts were subpleural, bordering on the pleura or on pleural adhesions (fig. 1). In both cases the subpleural involvement was most marked in the lower and central lobes and least in the upper lobes, where only the upper posterior portions contained a certain number of cystic areas; the apexes contained no cysts and showed no adhesions, while all the cystic areas were firmly attached to the parietal pleura. The mass of cysts progressed centrally from underneath the pleura, toward the hilus; the central portions, however, contained no cysts. The parenchyma where no cysts had yet developed showed certain microscopic changes of the alveolar tissue which indicated that the cystic lesions might ultimately have extended to the noncystic areas. The topographic arrangement of the cystic regions taken in conjunction with the fact that the alveolar apparatus as a whole was involved suggests that the extent of the respiratory movements had some bearing on the localization of the cystic changes.

Sclerotic changes in the lungs as well as in other organs in scleroderma have repeatedly been noted in the literature. Thus Nothafft [1] described increase of the interstitial fibrous tissue, and Kraus [2] reported a case of scleroderma in which chronic interstitial pneumonia was present. Murphy, Krainin and Gerson [3] reported a case in which the roentgen appearance of the lungs was suggestive of fibrosis. Weiss, Stead, Warren and Bailey [4] described fibrous lesions in the lungs and the myocardium. My case 2 presented, in addition to cystic lesions, extensive pulmonary fibrosis.

It is hoped that the present paper may help to clarify understanding of clinical conditions and roentgen appearances which in my case were interpreted as those of tuberculosis, probably owing to the peculiar disappearance of parenchyma. The 2 patients had been under the constant dermatologic supervision of Dr. A. Dostrovsky.[4a]

GROSS OBSERVATIONS ON LUNGS IN TWO CASES

CASE 1.—The patient was a man aged 23. The autopsy was made two and a half hours after death. The lungs, preserved in toto in solution of formaldehyde, presented the following picture: The volume of both lungs was reduced. The pleura was thickened, with remnants of fibrous adhesions, except for the apical and hilar regions; there were adhesions between the individual lobes and between the lungs and the diaphragm, those between the lobes occupying areas up to 2 cm. in diameter and being apparently of earlier date. Cysts of about pea size were seen shining through the thickened pleura.

The cut surface of a paravertebral section of the left lung showed in the lower lobe a cystic layer just underneath the pleura, 2.5 to 3.5 cm. wide, but narrower in connection with the diaphragmatic part of the pleura (1 to 2 cm). The borders between cystic and noncystic portions were sharp and jagged with small cystic protrusions advancing into the noncystic parenchyma. The central portion, i. e. the entire hilar region was free. The cysts were, as a rule, the size of a pinhead, intermingled with larger ones up to 1.5 cm. in diameter. The cysts were closely packed and separated from each other by narrow septums. The upper lobe, in which the apex was free from cysts (fig. 1), contained in its anterior portion patches of a narrow subpleural cystic layer, 0.5 to 0.6 cm. wide. Higher up these cystic patches were more isolated. A paravertebral section through the right lung showed the lower right lobe like the left. There was a peripheral layer of cysts—in part wider than that in the left; the majority were pea sized; a few were bean sized. The hilar region was free from cysts. The cystic areas were sharply outlined. In the upper lobe, apart from the apex, which was free from cysts, there was a peripheral cystic layer also spreading to the central portion. Striking was the extraordinary firmness of the cystic areas.

From the Department of Pathology, Hadassah University Hospital.

1. Nothafft: Zentralbl. f. Path. **9**:870, 1898.

2. Kraus, E. I.: Virchows Arch. f. path. Anat. **253**:710, 1924.

3. Murphy, J. R.; Krainin, G., and Gerson, M. J.: J. A. M. A. **116**:499, 1941.

4. Weiss, S.; Stead, E. A., Jr.; Warren, J. V., and Bailey, O. T.: Arch. Int. Med. **71**:749, 1943.

4a. Dostrovsky, A.: Arch. Dermat. & Syph., to be published.

CASE 2.—The patient was a woman aged 34. The left lung examined fresh was globe shaped, heavy; the pleura was attached in its lateral portions. Otherwise the pleura was smooth and shiny. On the paravertebral surface two parallel, quite superficial bands were seen translucent through the pleura, 1 to 2 cm. wide; on the cut surface these showed cystic structure. Apart from this cystic zone, everywhere on the cut surface much frothy fluid exuded on pressure; the parenchyma was throughout well aerated apart from the cystic zones, pieces of which sank in water. The lower lobe was markedly reduced in size and was firm and crackling to the touch; the cut surfaces were uniformly dark red; the lobe contained no air. In a layer 2 cm. in width the parenchyma was converted into small cysts of the size of a pinhead up to that of a small pea, with light gray, thin, shining walls; the rest of the parenchyma had a fleshy appearance. Specimens of both types of tissue, the cystic and the noncystic, sank in water.

The right lung was globe shaped, heavy; the upper and central lobes were elastic. The pleura showed thin adhesions in its lateral portions. The lower lobe was firm and crackling to the touch. On the surface the upper lobe was everywhere well aerated and on pressure copiously exuded a frothy fluid. The cut surface of the lower lobe was dark red and contained small cysts similar to those in the left lung, in cortical arrangement, but slightly larger than those in the upper lobe. The rest of the parenchyma gave a fleshy feeling to the touch. Specimens of all the portions sank in water. The small interpulmonary bronchi showed nothing abnormal.

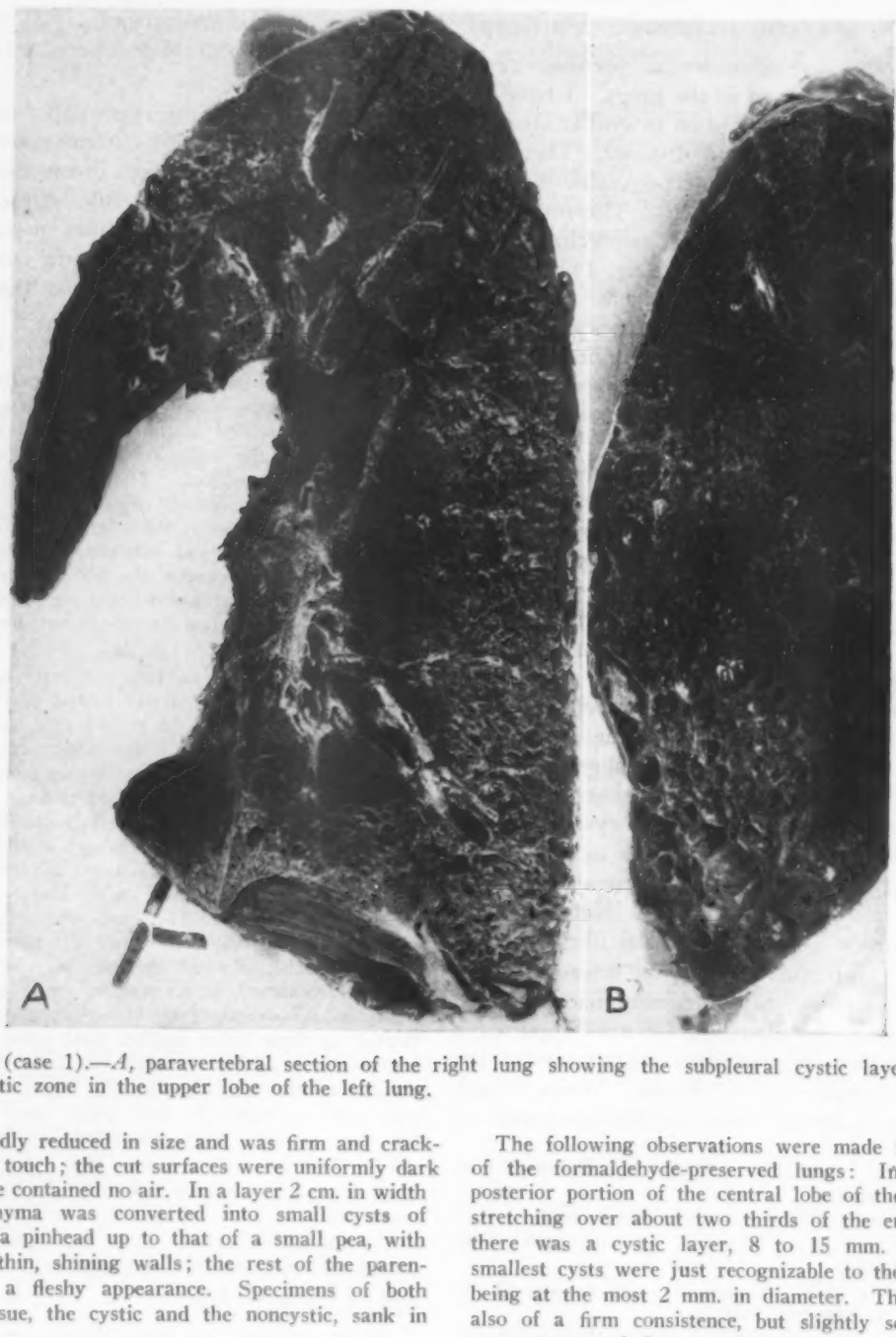

Fig. 1 (case 1).—*A*, paravertebral section of the right lung showing the subpleural cystic layer. *B*, subpleural cystic zone in the upper lobe of the left lung.

The following observations were made in the study of the formaldehyde-preserved lungs: In the upper posterior portion of the central lobe of the right lung stretching over about two thirds of the entire length, there was a cystic layer, 8 to 15 mm. wide. The smallest cysts were just recognizable to the naked eye, being at the most 2 mm. in diameter. This area was also of a firm consistence, but slightly softer in the greatest part of the central sector. In all other lobes the central part was likewise softer to the touch. The cystic portions were of firm consistence.

MICROSCOPIC OBSERVATIONS ON CYSTIC SCLEROSIS OF LUNGS IN BOTH CASES

The aspect of the cystic region was rather uniform. There were round, sharply outlined cavities (fig. 1) of varying size—from that of a pinhead to that of a pea, rarely more. No extremely large cavities were noted as they are encountered in other conditions characterized by cyst formation in the lungs as well as in other organs. The findings dealt with in the following paragraphs refer to case 1, supplemented by those of case 2. By careful study of the noncystic alveolar parenchyma it became possible to get an idea of the nature of the process that leads to cyst formation.

Noncystic Alveolar Parenchyma.—The alveoli were of moderate size or smaller, round and empty except for an occasional one containing old or recent blood with pigment-containing macrophages or erythrocytes (case 1). In case 2 the lungs showed in many areas the changes of catarrhal pneumonia with cellular desquamation and leukocytes but no intra-alveolar hemorrhages. The alveolar walls were somewhat thickened owing to marked congestion of the capillaries; the dilated capillaries did not protrude into the alveolar lumens as is the rule in chronic pulmonary congestion. The morphologic aspect of the diseased alveolar walls was rather varied but presented one common basis, viz., fibrosis. While normally only the so-called silver fibers can be made recognizable in the alveolar walls, fine collagenous fibrils were also demonstrable in both cases (Van Gieson; Mallory-Heidenhain stain); normal alveoli, i. e., with no identifiable collagenous fibrils, were exceptionally found in the apexes. The fibrils were here found in the intercapillary space, and the capillary wall was not involved in the process of fibrosis. The term "diffuse simple alveolar fibrosis" is proposed for the condition. On this simple alveolar fibrosis other fibrous changes involving the capillaries were superimposed, leading finally to disappearance of alveolar septums.

Rather frequently I saw fine hyaline or fibrous-hyaline portions of the walls containing neither blood vessels nor cells, preponderantly in the thin-walled alveoli, so that the parts of the wall involved had become even more slender, owing to hyaline metamorphosis (*a* in fig. 2 *A*). Usually the hyalinated parts occupied the whole stretch from one alveolar junction to the other, but sometimes the hyalination was restricted to shorter portions. These became attenuated in the middle, where rupture occurred (*b* in fig. 2 *A*). Hyaline stretches with interruptions were also encountered in parts where the alveolar walls showed marked fibrous thickening (*a* and *b* in fig. 2 *B*).

In the zones between cystic and noncystic parenchyma (case 1) there were long, slender hyaline or hyaline-fibrillar portions of septums containing neither blood vessels nor cells and showing peculiar degeneration eventually leading to marked extensive disappearance of the septum; they varied from pale reddish to gray and translucent in Van Gieson stains. The homogeneous septums appeared to be transformed into acollagenous structures as the result of some chemical process terminating in complete, diffuse dissolution of the whole part involved. Thus not infrequently there were rather long threadlike homogeneous septal portions, now more reddish, now more pale gray in appearance, representing the last remnants of the alveolar wall. In this way abrupt disappearance of considerable portions of the alveolar wall was brought about.

Fibrous-thready thicker stretches of septums, with peripherally situated nuclei and completely without blood vessels, consisted of collagenous fibrils in longitudinal arrangement within a pale yellowish substance (Van Gieson stain) in regular distribution. Peripherally there were a few, evenly distributed narrow oblong dark nuclei bulging into the alveolar lumen. The length of these stretches varied; the whole length of the alveolar septum between two junctions might be occupied or only part of it. Occasionally several of them occurred within one and the same septum. As a rare exception, one or another alveolus also showed diffuse changes of a similar character. Even in those fibrous-thready portions of the walls rupture was no rare event, owing to partial dissolution; the oblong pale tapering fragments, frayed out into a few fibrils at their extremities, were lying in a pale homogeneous substance. Here and there one found diffuse dissolution of the fibrous-thready portions; they were pale and appeared sometimes as though they were eroded—apparently in a state of diffuse dissolution.

Hyaline or hyaline-thready portions of the walls with preserved and not narrowed capillaries, both disappearing by autolysis (fig. 2 *C*), beginning with the capillaries, were encountered in a markedly hyperemic area, where the alveolar parenchyma was still present (case 2). Here and there the walls of every third or fourth alveolus were involved. There were long stretches (between two alveolar junctions or even far beyond) of hyaline or hyaline-fibrous changes in the walls, which were in a collagenous or acollagenous-hyaline state, pale gray (Van Gieson stain), apparently preliminary to dissolution. In figure 2 *C* one may note dissolution of the hyaline portion of the wall as well as of the blood capillary belonging to this particular portion. By far the most common picture was that of a minute part of a capillary broken off at both its ends and adhering to the hyaline wall. There was no accompanying hemorrhage. The erythrocytes contained in the capillary were enlarged and pale. It was apparent that circulation had ceased in such capillaries. In figure 2 *C* the capillary on the right, apart from the contours of its walls, was recognizable by the erythrocytes in it. On the left, although the contours were complete, the pale erythrocytes were recognizable only under the high power. Further to the left there were no remaining traces of the capillaries; the hyaline-fibrous portion of the wall had disappeared as though vanishing into a pale cloud of smoke.

The partial hyaline changes of the walls led to dissolution of the alveolar walls. By tears occurring in several adjacent alveoli small dissolution cysts developed, morphologically similar to emphysema (from substantive emphysema they are distinguished in the main by their hyperemia).

There were stretches of septums with pericapillary fibrils: On transverse section of the capillaries, the fibrils were occasionally seen to surround them circularly, and even where the capillaries were cut longitudinally fibrils sometimes formed an uninterrupted collagenous lining. The following changes were probably characteristic of the transition to collagenous portions which contained no blood vessels: A few of the alveolar walls were slender and contained many fibrils, capillaries being scarce and narrow and recognizable as such only by their containing erythrocytes, eventually disappearing altogether. They had scattered small dark nuclei. The destruction of the capillaries occurred as a result of the encroachment of surrounding connective tissue leading to obliteration.

Elastic fibers, which had disappeared wherever the alveolar walls were interrupted by fibrous-hyaline stretches, were also absent from the cyst walls. In the remaining alveolar parenchyma with diffuse simple fibrosis there were degenerative changes: The fibrils were slightly tortuous, frequently crumbling, with breaks

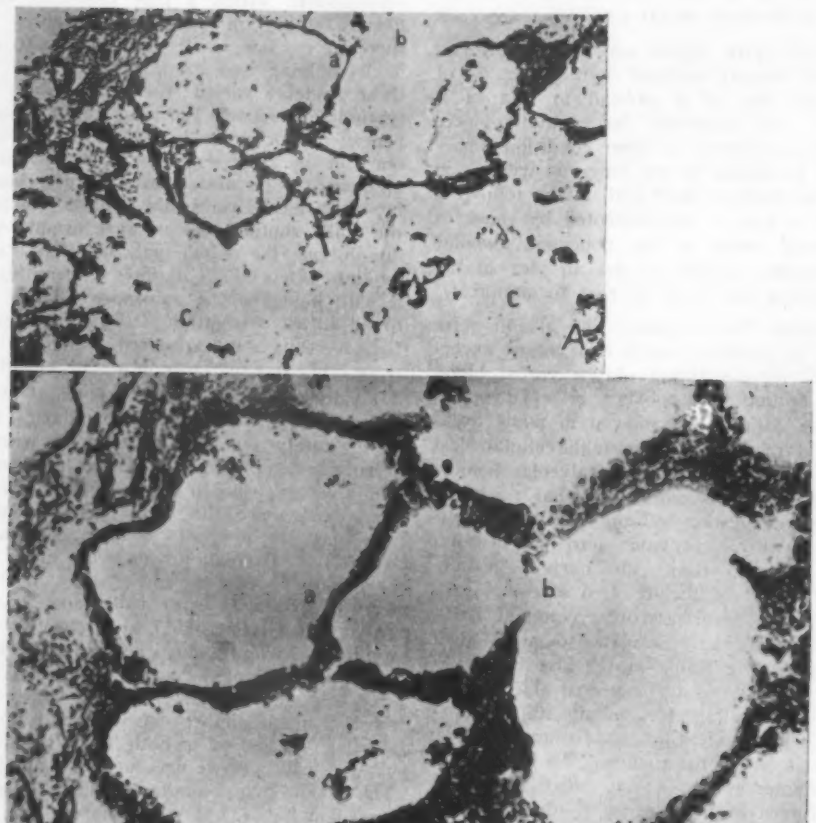

Figure 2

(See legend on opposite page)

in continuity. In areas of diffuse massive fibrosis (which will be described) elastic elements were entirely absent.

Outside the alveolar walls there was perivascular and pleural fibrosis, the latter occurring in the cystic areas (on the apical and hilar pleura without fibrous thickening). The alveoli adjacent to the perivascular fibrosis presented moderate atelectasis. The tissues with fibrous thickening, as far as this occurred outside the respiratory parenchyma—the perivascular and subpleural types—showed no degenerative changes, no absorption or atrophy of elastic elements, and the connective tissue of the bronchial ramifications and that of the interalveolar septums showed no degenerative changes. The not involved collagenous framework of the lungs appeared to form a barrier against further progressive distention of cysts so that their size could exceed a certain limit.

Should the alveolar process be considered peculiar to my cases of scleroderma? To form an opinion on this problem I selected at random various pulmonary conditions (including substantive and vicarious emphysema of various origin, at different ages, from a few days up to old age). Septal portions of a hyaline or hyaline-fibrous nature with no capillaries were encountered under the most varying conditions, and even alveolar tears were rather frequently found. Thus in lungs presenting all kinds of influenza pneumonia one sees groups of empty alveoli surrounded by solidified pneumonic parenchyma, alveoli with extensive hyaline degeneration of the walls and even tears. A similar picture is presented in lungs showing vicarious emphysema associated with pertussis pneumonia. In essential emphysema the tears occur in those portions of the wall which contain no capillaries and show a hyaline or hyaline-fibrous structure. The findings presented by the lungs of a 17 day old child with catarrhal pneumonia associated with generalized edema of the skin were interesting, because changes of this type were rather frequent. As the same histologic picture is encountered in a variety of diseases, the question may arise whether the elimination of pulmonary alveoli by hyaline metamorphosis of their walls may signify—within certain limits—a pathologic physiologic process. The other fibrous types referred to in the foregoing pages and further simple diffuse fibrosis of the walls, as well as the massive type (which will be referred to), were absent in these controls.

Cystic Parenchyma.—In the peripheral zones, the alveolar parenchyma had completely disappeared, and the fibrous interstitial tissue was abundant. In the internal zones of the cystic areas there were left small remnants of the alveolar parenchyma.

The peripheral zones were largely composed of big roundish cysts with reduced wall tension, not infrequently with a short, narrow branch. While the epithelium of the main cavity might be of varying character even within one and the same lining, the narrow ramification might show a diffuse uniform layer of small cuboidal cells. On the whole, the epithelium was arranged in one layer presenting cells of the cuboidal or short columnar, occasionally of the high columnar, type. As a rare exception, one found a short length of ciliated epithelium. The epithelium lay on a fibrous layer, and no basement membrane was recognizable. There was no muscular layer or cartilage. Some of the cavities showed a uniform epithelial lining, while in others the character of the epithelial cells varied. There were also spaces with a great number of detached epithelial ribbons, occasionally filling up the entire lumen. A peculiar uniform type of epithelium was sometimes found lining an oblong or round, smaller or larger cavity: The epithelial bodies were oblong, tapering toward the end to the thinness of a thread (spindle shaped); the comparatively large globular nuclei were situated in the center, bulging toward the lumen and surrounded by a narrow seam of protoplasm. Where the epithelial layer had become detached in toto it formed chains of spindle cells. Some of the oblong cavities were packed with desquamated ribbons of spindle cells. This epitheluim resembled the swollen respiratory epithelium of alveoli or alveolar ducts described by Miller.[5] In my material I further found more recent stages of epithelial swelling: The cells were of the small cuboidal type, with large globular nuclei markedly protruding toward the lumen, lying close together; where a ribbon of these cells was detached it appeared like a string of pearls. The peculiar character of this epithelial lining indicates that the structure of these cavities followed the type of respiratory parenchyma.

Other areas of the peripheral zone, apart from the rounded cavities, consisted in the main of ductlike spaces of varying length and width, apparently following no special law as to ramification and epithelial lining; some were y shaped or h shaped; some had irregular ramifications in all directions. Striking was their extraordinary length as compared with their width; some of these corridors stretched over two to three low power microscopic fields, with lumens narrow, measuring about 5 to 10 microns in width. As an appendage to almost every one of these long corridors I found small roundish cavities, probably communicating with each other (rudimentary alveolar structures?). There were further narrow, perfectly straight ductlike formations extending over one to two low power microscopic fields with no ramifications. Their lining showed varying aspects: high-columnar epithelium or chains of spindle cells—here occasionally with a rich subepithelial network of replete blood capillaries, following the alveolar arrangement. In the middle of a large compact fibrous field (fig. 3*C*, the dark section) there were numerous

5. Miller, W. S.: The Lung, Springfield, Ill., Charles C Thomas, Publisher, 1943.

EXPLANATIONS OF FIGURE 2

Fig. 2 (case 1).—Disappearance of alveolar walls. *B* and *C* show for the most part alveolar walls containing many cells. In *A* one may see: (*a*) a hyaline portion of wall just about to disappear; (*b*) a defect of wall, with a hyaline tapering remnant of wall to the right; (*c*) areas with almost complete disappearance of alveoli and the presence of small cysts with no proper wall ("dissolution cysts"). In *B* there may be seen (*a*) a hyaline stretch of wall and (*b*) a wall defect due to disappearance of a hyaline stretch; below one sees a tapering remnant of the hyaline stretch of wall (similar to that in *A*). *C*, an alveolus with a hyaline-thready, thickened stretch of wall (marked by arrow upward at center) which on the left seems to vanish into a cloud of smoke. An accompanying capillary (downward arrow) may be seen on the right, containing swollen, closely packed erythrocytes; on the left the capillary is just about to disappear but is still recognizable by its contours and the pale erythrocytes, while still farther to the left it has disappeared. The dark-stained portions of the wall contain rather a large number of cells and fragments of capillaries. At three points (marked *a*) the innermost part of the wall shows fibrosis and contains no capillaries.

perfectly straight slitlike corridors—appearing as though scratched with a needle on a firm material—lined with short columnar epithelial cells. The scarcity of cystic distention in these parts indicates that growth had been limited by the already consolidated fibrous character of the locality.

In the internal zones of the cystic areas a particularly instructive aspect was provided by the small cyst areas protruding into the still noncystic parenchyma which already showed signs of future cystic development. In places the cavities showed sharp outlines and notable. In spite of the absence of histologic evidence, the existence of some biodynamic relationship between them was to be assumed. Further analysis of the entire material revealed that the disappearance of parenchyma was accompanied by bronchiolar proliferations which later underwent cystic distention. Simultaneously with the disappearance of parenchyma, fibrous tissue was being established, which was subsequently invaded by bronchiolar proliferations. I should like to call this process "cystic bronchiolar hyperplasia." Proliferation here was extraordinarily variegated in its appearance,

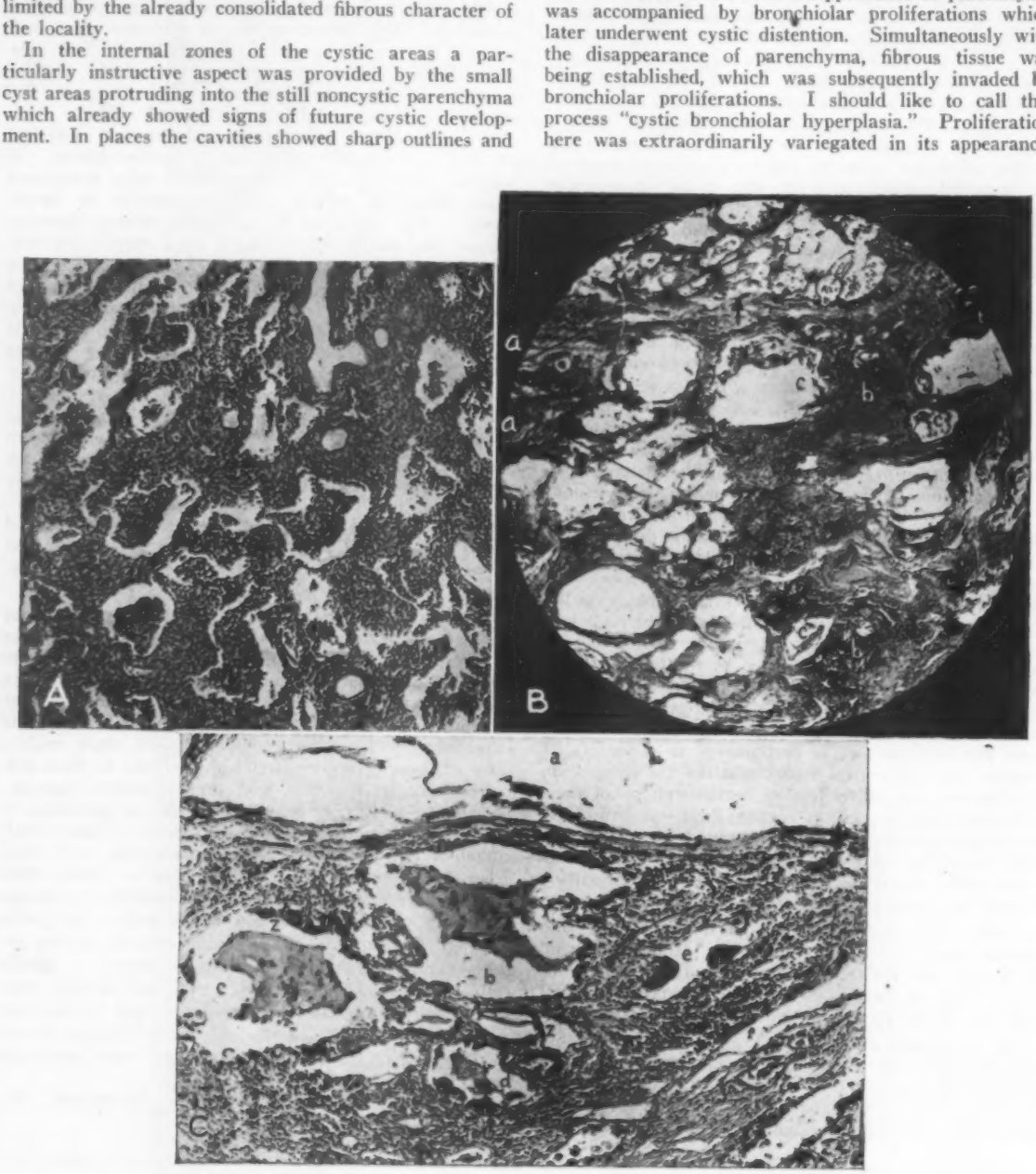

Fig. 3.—*A*, marked interalveolar fibrosis (so-called interstitial pneumonia); the alveolar spaces contain desquamated cells and leukocytes. In *B* at two places there are alveolar remnants of parenchyma with extensive dissolution (marked by pointers *a*, *a*; both areas are indicated also by arrows). The darker areas on the right (*b*) consist of lymphoid tissue, with fine gaps representing recent bronchiolar proliferations. *C* shows cysts of varying size, the lining epithelial cells being partly of the high columnar type (*z*). In the largest cavity (*a*) the epithelium together with the mucous matter has become detached. At *b*, *c* and *d* the mucous matter is intermingled with large cells with round nuclei; *d* and *e* are padlike bronchiolar proliferations. Solid islets of epithelium are seen to the right (*d*).

microscopically an epithelial lining. In the adjacent, noncystic areas one found the aforementioned hyaline stretches of alveolar walls with dissolution. The absence of transitional stages between the spaces of dissolution of parenchyma and the epithelium lining cysts was in the initial stage as well as in the cystic stage. In close proximity to islets of recognizable alveolar parenchyma with dissolution spaces there might be numerous cysts of varying shape lined with epithelium sending off lateral sprouts of even more recent date.

Certain aspects repeated themselves, e. g., bottle-shaped cavities with narrow lateral sprouts at their necks and biscuit-shaped cavities with bilateral narrow branchings at either extremity. Within areas of preserved alveolar parenchyma other sections from the same block revealed bronchioli which grew longitudinally; apart from fragments of elongated and distended alveolar bronchioles there were stretches of bronchiolar formations presenting various types of ramifications. Sometimes one saw a long, wide "corridor" with dichotomic ramifications of a slightly smaller caliber, which, in turn, sent off even narrower branches in which the lumens were just barely recognizable. Striking was the variety in the type of epithelial cells, particularly the sudden change from the high columnar to the low cuboidal type. In close proximity to the ductlike formations there were frequently small, narrow cavities within lymphatic tissue, of recent date.

One of these ramified bronchiolar formations was joined by a peculiar adenoma-like complex, consisting of closely packed roundish cavities, about the size of alveoli, filled with a mucous matter which stained blue with hemaium. The cavities constituting the pseudo-adenoma partly communicated with each other or—as in some of the sections—communicated with the duct. The epithelium varied in cell type from the flat and small cuboid type to the high columnar, the latter occurring over short stretches. Similar cavities, singly or in batches, were also found higher up the duct or farther off. They were easily recognizable even under low magnification by their characteristic contents staining blue with hemalum. The same sort of cavities, or slightly wider and rounder ones, were found in sections of almost every block, containing the same matter, which, however, sometimes showed marked retraction from the cyst wall. They also appeared singly or in small batches of several. The peculiar mucous material contained in the cavities included unevenly scattered, rather large rounded cells with vesicular nuclei. Some of them had a jagged appearance. The further development of the jagged structures was revealed in case 2, in which definite ossification of the contents of similar cavities could be established. As a rule the latter type appeared singly or in small groups. In case 1 the initial stage, in case 2 the complete stage of ossification (of a lamellated structure), was seen. The bronchiolar epithelium produced the mucous material; ossification occurred as a result of immobilization. The mucous material was encountered in the ducts; it showed no ossification.

Initial stages of bronchiolar proliferation appeared in a great variety of shapes: First may be mentioned hollow formations in continuation of existing ones (as was seen in a number of the photomicrographs). Interesting were numerous discrete hollow sprouts developing in this way, which ramified within the thickened pleura. There were widespread small cavities within the lymphatic tissue. Often striking was their great number; they had a regular epithelial lining, which consisted of small cuboid or columnar cells or epithelial spindle cells. Second, there were solid epithelial accumulations in the neighborhood of small cysts or there were solid epithelial pads, one to three, together in one small round cavity; sometimes a cavity was completely filled with these epithelial accumulations. Such epithelial pads have been described in the thyroid gland, later developing into the thyroid vesicles.

In the peripheral zones there were further, recently formed sprouts of cavities. Here and there one noted lymphatic foci containing young cavities lined with epithelium.

The extensive proliferations must, therefore, to all appearance, be considered a response to the disappearance of alveolar tissue (or is one allowed to speak of alveolar regeneration?). In any case, the disappearance of alveolar tissue was comparable to multiple amputations occurring at the extremities of the bronchial tree. Although some of the newly formed cavities showed certain characteristics of pulmonary alveoli—spindle cell chains lining the lumens, an extraordinarily rich network of wide, replete subepithelial capillaries—I did not find all these characteristics together in one and the same formation. Thus I saw in my cases, in spite of extensive proliferation, no formation of regular respiratory alveolar parenchyma.

A fair-sized accumulation of small cysts, the whole approximately 1 cm. in diameter, consisting of alveolectasis, was seen in case 2; in the microscopic pictures the alveolar walls show thickening and contain an abundance of collagenous fibrils. By the rigid, gaping character of the cavities these areas may, in a fresh state, be distinguished from those of "ordinary," i. e. the classic type, of pulmonary emphysema.

The following paragraphs contain descriptions of fibrous islets from which the alveolar parenchyma had completely disappeared, which were accompanied by areas of small cysts constituting a fibrosed type of emphysema.

MICROSCOPIC OBSERVATIONS ON COMPACT PULMONARY SCLEROSIS IN THE SECOND CASE

In case 2 compact sclerosis was present extensively in the two lower lobes, which had the gross appearance of carnification; it was also present in the middle lobe, but was absent in the upper lobes. One is dealing here with areas of almost complete solidification, frequently closely packed, nodular or elongated, and varying (fig. 3) in size from 1 to 15 mm. In numerous places it was possible to follow the way in which fibrosis had occurred. The initial stage may be characterized as "diffuse massive fibrosis of the alveolar walls"—a process distinguished by its progressive nature. The alveolar walls became thickened to almost twice their original size or even beyond that. The capillaries were narrowed, circular or oblong, with cellular desquamation and were eventually recognizable as alveoli only by a few desquamated cells in the fibrous mass or even disappeared without leaving any trace. There was thus eventual disappearance of parenchyma as a result of progressive fibrosis of the walls (with disappearance of the capillaries) without dissolution. The specific character of the alveolar walls themselves was, as a rule, no longer recognizable either in part or along the entire wall. The appearances might indicate a slightly thickened alveolar wall containing a fair amount of capillaries, but apart from this the lumen bordered on a large diffuse fibrous interalveolar mass. A formation which originally must have been an alveolus was found as an empty space enclosed by a fibrous mass, occasionally with cellular desquamation. Frequently fibrous islets were in close proximity to areas of small cysts. These may be considered as patches of compensatory emphysema, viz., fibrosed emphysema.

The sclerosis of the pulmonary tissue further manifested itself by interalveolar fibrosis. A slight degree was encountered in case 1 in some places; a higher degree, with preponderance over still existing alveoli, in case 2. The interalveolar fibrous tissue was of moderate cell content, containing oblong cells with vesicular nuclei.

Peribronchial adenomatous zones were noted within the fibrous patches after disappearance of the alveolar parenchyma. Now and then there was a communication between the central bronchus and one of the cavities of the peribronchial formations. Occasionally two or three of these peribronchial zones lay together, thus creating large adenomatous areas. Sometimes the original bronchus had a muscular layer which was traversed by the newly formed cavities. A few of these newly formed cavities were embedded in lymphatic tissue. Within the dense fibrous patches the bronchiolar proliferations did not become subject to cystic distention.

SUMMARY

Two cases of scleroderma presented disappearance of alveolar tissue in the lungs as a result of two distinct processes: lysis of the alveolar walls and progressive sclerosis.

Cystic pulmonary sclerosis occurred on the basis of a hyaline or hyaline-fibrous process involving the alveolar walls, accompanied by disappearance of capillaries and superimposed on generalized diffuse simple fibrosis of the alveolar walls; after dissolution of the hyaline portions of the walls the process led to disappearance of alveolar parenchyma (fig. 2). After a preliminary fibrous stage the alveolar parenchyma was replaced by cystically enlarged bronchiolar proliferations, a process for which the name "cystic bronchiolar hyperplasia" is proposed. The cysts establish themselves in the subpleural zones except in the apexes and the hilar regions (fig. 1 *B*).

Compact pulmonary sclerosis developed on the basis of diffuse compact alveolar fibrosis of progressive nature with disappearance of capillaries and parenchyma, owing to progressive narrowing of alveoli. There was no dissolution. The capillaries persisted and with them the alveoli, and extensive interalveolar fibrosis developed. The bronchi and bronchioli which remained intact in the fibrous areas were surrounded by peribronchial proliferations forming adenoma-like zones.

MIXED TUMORS OF THE SKIN

REPORT OF CASES, WITH A CONSIDERATION OF THE HISTOGENESIS OF MIXED TUMORS OF ORGANS DERIVED FROM THE ECTODERM

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The mixed tumors are a poorly defined group neoplasms composed of two or more types of ssue and characterized by their pleomorphism. hey are found in many situations in the body d have been attributed histogenetically to each the three germinal layers. Those originating structures derived from the ectoderm most en arise in connection with the salivary glands, he great majority of them being situated within ese structures or in close proximity to them.

Occasionally neoplasms which are morphogically identical with mixed tumors of the alivary glands arise in the skin, in the mucous nembranes and in glandular structures other han the salivary glands. They are most comonly located in the cephalic region in close roximity to embryonic fissures, the lips,[1] the buccal mucosa,[2] the palate[3] and the lacrimal glands[4] being the most frequent sites of origin.

Cutaneous mixed tumors are rare. Such mors have been seen, however, on the scalp[5] nd the face[2] and in rare instances on the forerms,[6] the hands,[7] the thighs[8] and the legs.[6] Neoplasms of this type arising on the trunk have ot been reported.

Scharla[9] reviewed the literature in 1926 and as able to find only 5 cases of extracephalic cutaeous mixed tumors recorded; to these he added 1 case of his own. Two years later Simard[7] reported an additional case and called attention to Hirsch's[8] case, which Scharla had overlooked. Both failed, however, to include a report by Geschickter and Koehler,[10] in which 2 cases were described under the heading "Aberrant Salivary Tumors." Gates, Warren and Warvi[11] have recently reported a mixed tumor which originated from the skin of the hand.

Interest in these heterotopic neoplasms lies not only in their rarity but in the opportunity they afford to study the histogenesis of this rather bizarre group of mixed tumors of the salivary gland type. The material for the present study consisted of 4 cutaneous mixed tumors located on the skin of the breast, the nose, the sternum and the ear. The tumors arising in the skin of the breast and the sternum are of peculiar interest, since, so far as I know, they represent the first reported neoplasms of this type which originated within the skin of the trunk.

REPORT OF CASES

CASE 1.—A 64 year old Negro school teacher consulted Dr. N. P. Battle, of Rocky Mount, N. C., on June 29, 1943, because of a small nodule in the right breast. She stated that approximately ten years previously she had felt a stinging sensation in this organ, which she attributed to the bite of an insect. On examining the breast, she noticed a wheal which presented a reddened surface. The lesion gradually increased in size and became reddish brown.

The physician found the left breast to be without evident lesion. Involving the skin of the right breast in the lower right quadrant just external to the median line was a spherical mass which was freely mobile and appeared to be approximately 1.5 cm. in diameter. The overlying epidermis was pigmented, and the mass felt cystic. Lymph glands were not palpable in the axilla. Under local anesthesia the tumor was completely excised without difficulty.

Gross Description.—The excised specimen consisted of an encapsulated firm nodule, which was spherical in shape and measured 1.5 cm. in diameter. The cut surface was grayish white and resembled cartilage. Surrounding the tumor was a small amount of fibrofatty tissue, which on one surface appeared to be loosely attached to the skin.

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From the Division of Pathology of the Department of Pathology and Bacteriology, Bowman Gray School of Medicine of Wake Forest College, and the Baptist Hospital.

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7. Simard, L. C.: Am. J. Cancer **33**:182, 1938.
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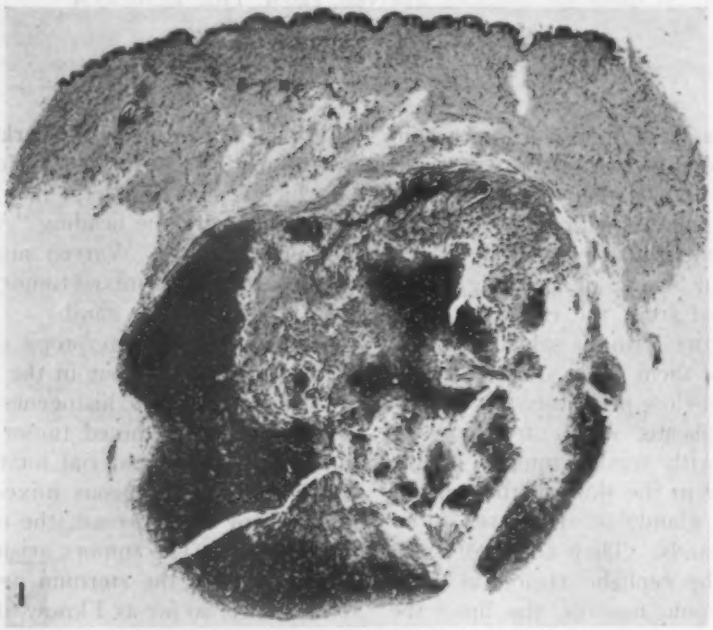
10. Geschickter, C. F., and Koehler, H. P.: Am. J. Cancer **23**:804, 1935.
11. Gates, O.; Warren, S., and Warvi, W. N.: Am. J. Path. **19**:591, 1943.

Microscopic Description.—The tumor was roughly oval in shape and was situated within the corium (fig. 1). With the exception of the superior surface, it appeared to be completely surrounded by a delicate capsule, which contained atrophic sweat glands. The superior surface of the tumor appeared to be continuous with the corium, from which coarse strands of collagen penetrated into its substance (fig. 2). On this surface the cutaneous appendages were perfectly normal, and a few fat cells, together with a single section of a sweat gland, were seen in one area.

The capsule was thin and was composed of loose connective tissue fibrils interspersed with dense strands of collagen which radiated in all directions. The fibrils appeared to represent the cytoplasmic processes of stellate and spindle-shaped cells and were continuous with identical fibrils located in the substance of the tumor. In sections stained with Mallory's aniline blue and counterstained with orange G, the nuclei of these cells appeared reddish blue in some instances and glassy red in others. Many mast cells containing round or oval granular nuclei and completely surrounded by large



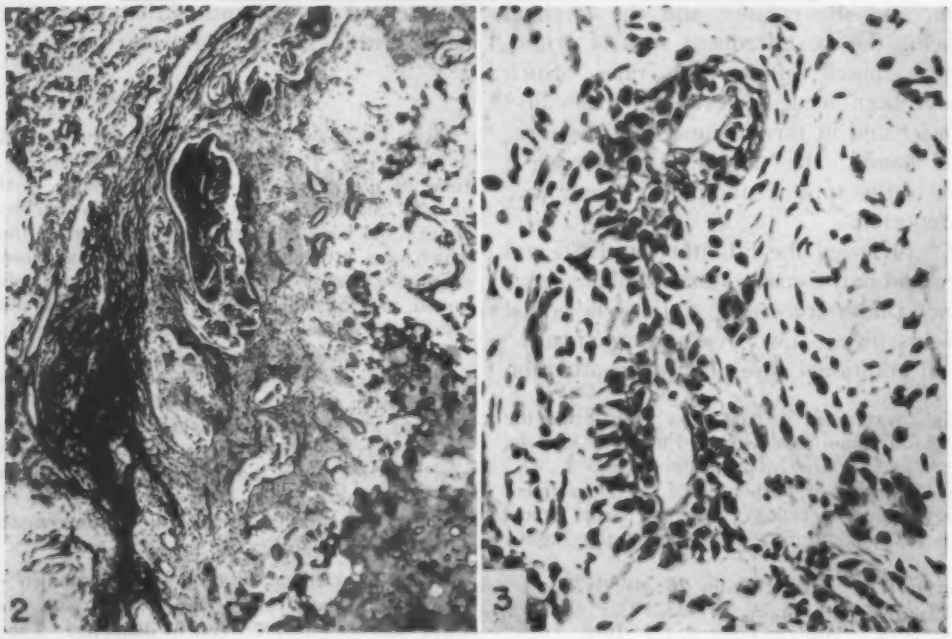


Fig. 1 (case 1).—The entire tumor is shown. × 8.

Fig. 2 (case 1).—Bands of collagen can be seen extending from the capsule into the substance of the tumor. Cysts are present, which are filled with an amorphous granular material. × 60.

Fig. 3 (case 1).—Myoepithelial cells at the periphery of the epithelial collections show marked proliferation. × 320.

numbers of purple and glassy red granules were seen in these preparations.

The tumor proper was composed of columns, islands and tubuloalveolar collections of epithelial cells, which were surrounded by loose connective tissue and interspersed with large amounts of cartilaginous tissue. The epithelial collections were scattered throughout the amorphous bluish granular material (fig. 2). Certain cysts and portions of others were lined by a double layer of flattened, atrophic cells. Other areas in the tumor presented prickle and myoepithelial cells, which appeared to be interspersed between fibrils and collagenous bundles (fig. 3). These epithelial cells were morphologically identical with the cells which normally

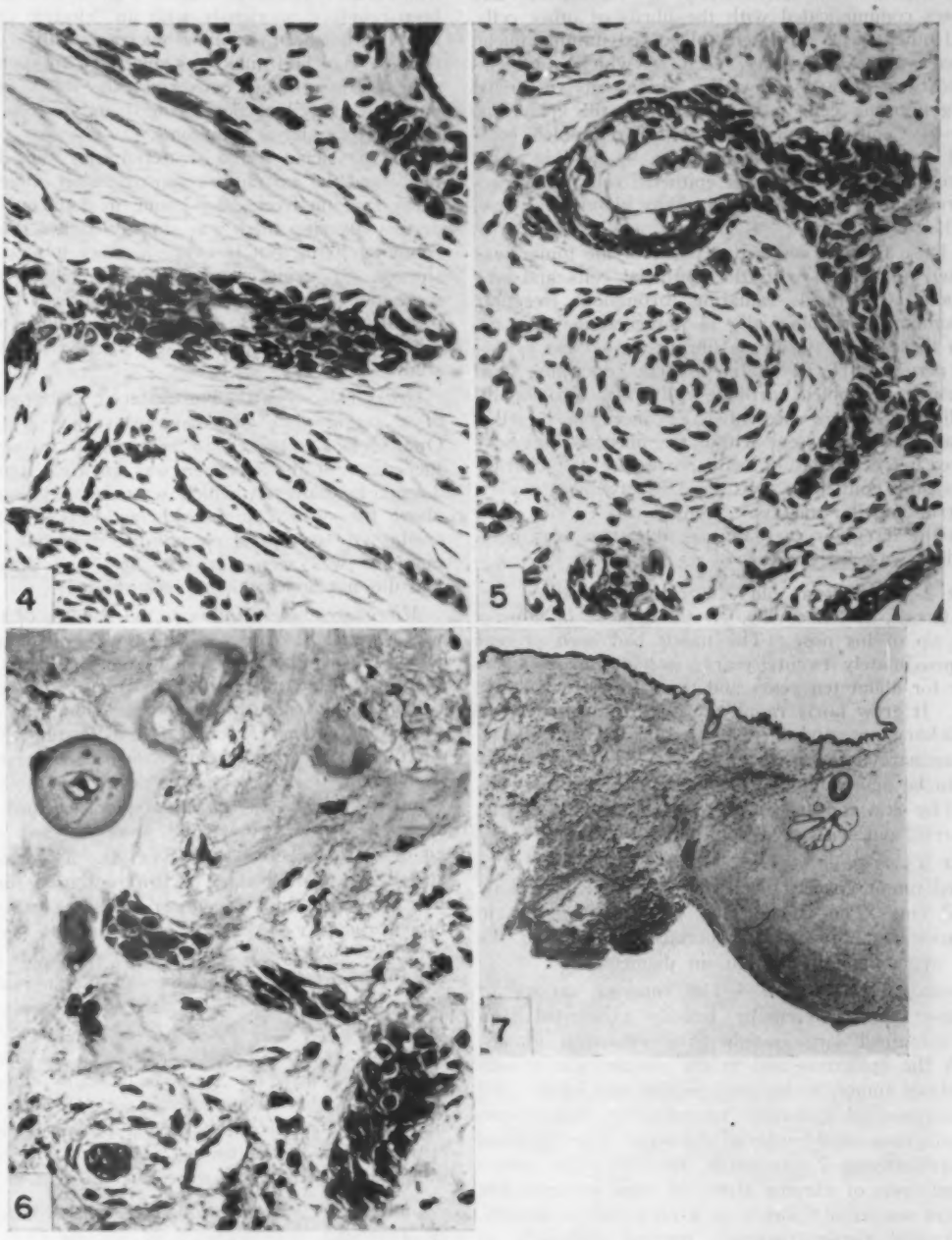


Fig. 4 (case 1).—In the center of the photograph an epithelial process is seen which resembles an ectodermal primordium. Beginning cavitation is present in its center. × 320.

Fig. 5 (case 1).—Some of the cells resemble those which form sebaceous glands. In the center of the photograph a miliary myxoma is seen. × 320.

Fig. 6 (case 1).—Groups of epithelial cells and individual epithelial cells are intimately associated with the formation of fibrocartilaginous areas. × 320.

Fig. 7 (case 3).—A portion of the tumor is seen with its surrounding structures. × 8.

tumor but were most numerous in the region of the dermis. Numerous cysts were seen near the superior surface of the neoplasm, and these were filled with an

constitute the prickle layer of the skin, and intercellular bridges, epithelial fibrils and keratohyaline granules were clearly discernible. Vacuolated spaces fre-

quently appeared between the cells and in some instances appeared to separate them entirely from the parent cellular mass. In preparations stained with Mallory's aniline blue and counterstained with orange G, the individual cell consisted of a homogeneous bright red nucleus surrounded by numerous fibrils. In some instances the latter ended abruptly in clear spaces and in others communicated with the fibrils of other cells. In still other areas shrunken, folded red-staining nuclei occupied vacuolated spaces, each of which was surrounded by a distinct cell wall. These cells resembled the degenerating cells normally seen in the epidermis. Finger-like epithelial processes which resembled ectodermal primordia were present (fig. 4), and in some instances differentiation of the epithelial cells into structures resembling sebaceous and sweat glands was seen (fig. 5).

The loose fibrillar connective tissue of the tumor was intimately associated with the epithelial cells and was continuous with fibrils radiating throughout irregular areas similar morphologically to fibrocartilage (fig. 6). Within these areas the dense fibrous tissue was everywhere permeated by a hyaline matrix containing oval cells which resembled cartilage cells, and occasionally the adjacent matrix presented a concentric lamellar appearance. Also present within the fibrocartilaginous-like area were whorls of cells possessing the characteristics of epithelial cells and surrounded by a concentric lamellar matrix. Definite mast cells were seen in the irregular cartilaginous-like areas, and perichondrium was not present.

Case 2.—A 69 year old Negro man consulted Dr. T. V. Goode, of Statesville, N. C., because of a mass on the tip of his nose. The tumor had been present for approximately twenty years; it had remained stationary for about ten years and then began to increase in size. It grew fairly rapidly until it attained the size of a hickory nut, and then again remained stationary. On examination, the mass on the tip of the nose was found to be approximately 3 cm. in diameter. It was covered by skin and appeared to be lobulated. Excision was carried out without difficulty.

Gross Description.—The specimen consisted of a lobulated tumor covered by skin and measuring 2.5 by 2 by 2 cm. The cut surface was cartilaginous in appearance and presented numerous small cysts, the largest approximately 0.9 cm. in diameter.

Microscopic Description.—The superior surface of the tumor was covered by heavily pigmented skin which contained large numbers of sebaceous glands. Beneath the epidermis and in the corium was a well encapsulated tumor, which was divided into lobules and was composed of epithelial, myxomatous, chondromatous, collagenous and lymphoid elements. The epithelial cells were arranged into cords, tubuloalveolar collections and cysts of varying sizes; in some instances the cells were scattered diffusely in a myxomatous stroma. This stromal tissue frequently merged insensibly to form irregular areas which resembled cartilage but which were not surrounded by perichondrium. The epithelial cells in certain localities appeared to differentiate into cells resembling those which normally form the prickle cells of the epidermis, and occasionally one could see epithelial pearl formation. Differentiation of the epithelial cells into tubuloalveolar structures bearing a superficial resemblance to sweat glands was also noted. This microscopic picture, although it closely resembles that observed in case 1, differs from it in certain particulars.

Case 3.—A 38 year old white woman consulted Dr. H. H. Bradshaw, of the North Carolina Baptist Hospital, Winston-Salem, on Oct. 5, 1944, because of multiple small nodules in the skin of the trunk and the extremities. The nodules had been present for two years, and she had had no symptoms except those of tumor. For cosmetic reasons four of the nodules had been removed previously with an "electric needle," but they had not been examined microscopically. She stated that she had had numerous small brown spots over the entire surface of her body since she was 1 year of age but that they had not changed in size or color nor had they produced symptoms.

The physician found scattered over the skin of the trunk and the extremities approximately fifteen elevated areas ranging from 2 to 3 mm. in diameter. The epidermis covering them was slightly darker than the surrounding tissue, but in every instance it was intact and showed no evidence of inflammation. The nodules were not attached to the underlying tissues but appeared to be a part of the skin. Under local anesthesia one of these nodules was excised for microscopic study.

Gross Description.—The material submitted consisted of a single piece of tissue measuring 2 by 0.5 cm. One surface appeared to be covered by skin. Near the center of the specimen was an area approximately 3 mm. in diameter, which was elevated about 1 mm. above the surface of the skin and was slightly roughened. On section a circumscribed nodule 3 mm. in diameter was seen. It was firm and grayish white and did not appear to be encapsulated.

Microscopic Description.—One surface of the section was covered by stratified squamous epithelium, beneath which the papillary layer of the dermis could be clearly seen. The latter tissue merged with the reticular or collagenous tissue, which contained numerous sweat and sebaceous glands. A lobulated and circumscribed tumor surrounded by collagenous bands was present in the dermis (fig. 7). The greater portion of the tumor was composed of loosely arranged spindle-shaped cells possessing delicate cytoplasmic processes and resembling embryonic connective tissue (fig. 8). These cells represented, however, a stage of differentiation further than that seen in the loose connective tissue stroma of most mixed tumors. In the substance of the tumor were epithelial cysts, lined by stratified squamous epithelium and containing an amorphous, acellular material, together with well differentiated sweat and sebaceous glands (fig. 9). In some areas structures resembling embryonic sweat glands were seen and were surrounded by connective tissue which was more myxomatous than that seen in the greater portion of the tumor. Fat cells of the adult type were seen in certain portions of the tumor. Whorls of tissue resembling miliary myxoma were also present.

Case 4.—A 53 year old white woman consulted Dr. A. Papineau, of Plymouth, N. C., on Oct. 12, 1943, complaining of a tumor on the superior surface of the auricle. The patient did not know the exact duration of the growth but stated that it had been present for more than ten years. It had grown slowly, and there had been no increase in size for several years. Examination showed a circumscribed nodule approximately 1 cm. in diameter which appeared to be a part of the skin. The tumor was excised without difficulty.

Gross Description.—The specimen consisted of a small nodule, which was oval in shape and measured 8 mm. in diameter. One surface was covered by skin.

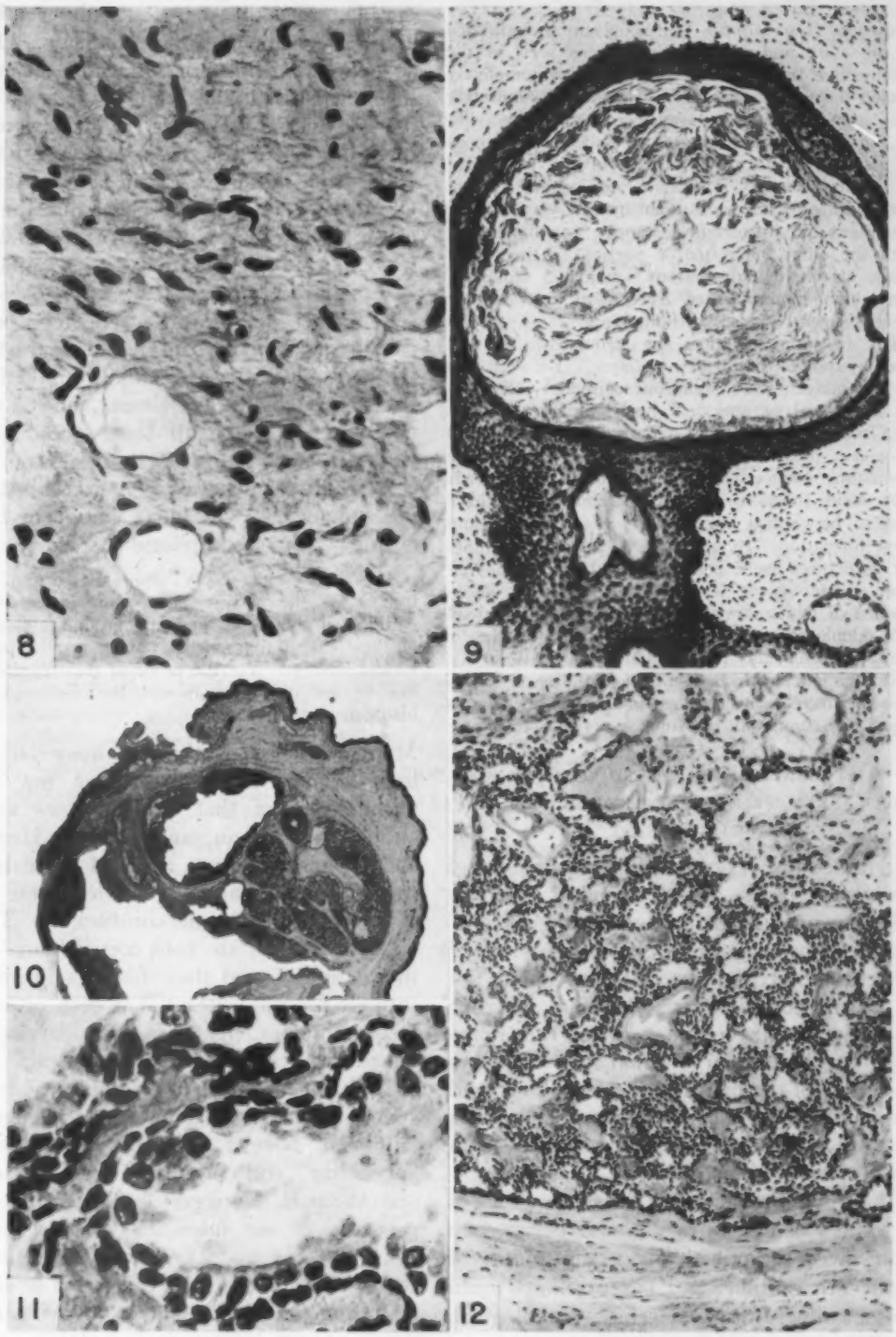


Fig. 8 (case 3).—Loosely arranged connective tissue is interspersed with fat cells. × 589.

Fig. 9 (case 3).—This is an epithelial cyst surrounded by young connective tissue cells. Sebaceous cells can be seen in the lower portion of the photograph. × 110.

Fig. 10 (case 4.—The entire tumor is shown. × 8.

Fig. 11 (case 4).—Alveolar spaces are seen lined with tall cuboidal epithelial cells. Myoepithelial cells surround the cubical epithelium. × 580.

Fig. 12 (case 4).—Cylindromatous hyalinized bands of collagen separate groups of epithelial cells. Myxomatous connective tissue can be seen in the upper portion of the photograph. × 110.

Beneath the skin was an irregular grayish white area which was very firm and contained numerous cystic spaces.

Microscopic Description.—The neoplasm was situated within the skin, and sweat glands were seen in the loose connective tissue of the corium, which completely surrounded the tumor (fig. 10). The major connective tissue septums of the neoplasm were continuous with similar bundles in the corium and subcutaneous connective tissue, and fat was present in the peripheral portion of the tumor. The tumor presented distinct nodules separated from each other by heavy connective tissue septums or, in certain instances, communicating one with the other. The major portion of the tumor was made up of cylindromatous hyalinized bands of collagen which communicated freely with one another and were seen in both longitudinal and cross section (fig. 12). In some instances the stroma was formed of loose myxomatous connective tissue. Between the bundles of collagen were numerous epithelial cells possessing large oval or spherical nuclei and scant cytoplasm. Occasionally the cells were more polygonal and the cytoplasm more abundant. In some instances the cylindromatous connective tissue processes were separated by solid sheets of cells, while in others there appeared to be central degeneration of the epithelium with cyst formation. In other areas, however, definite alveolar structures were present (fig. 11). Occupying the large cystic spaces was a finely granular amorphous material which contained an occasional red blood cell. A double layer of cells limited peripherally by a hyalinized membrane was present in certain areas. The histologic picture was identical with that of neoplasms which have been described in the salivary glands and which are commonly classified as cylindroma.

THE HISTOGENESIS OF MIXED TUMORS OF THE SKIN, THE SALIVARY GLANDS AND THE BREAST

Since mixed tumors of the skin, the breast and the salivary glands appear similar morphologically, it would seem that mixed tumors arising in these organs should be closely related histogenetically, regardless of their point of origin. Any consideration, therefore, relative to the origin of mixed tumors arising in organs derived from the ectoderm would logically begin with a review of the present knowledge of mixed tumors of salivary glands. These more common neoplasms have been extensively studied from the point of view of histogenesis.

The controversy concerning the histogenesis of mixed tumors of the salivary glands began soon after they were described, about the middle of the eighteenth century, and has continued until the present time.[12] Early German pathologists, led by Billroth, considered these tumors to be of mesenchymal origin, while Robin and others of the French school considered them to be epithelial in nature. The theory that they were derived from lymphatic endothelium was introduced toward the end of the nineteenth century and was championed by Volkmann. However, as a result of Hinsberg's attack on the endothelial theory in 1899, the tendency during the next few years was to return to the original views regarding the histogenesis of these tumors. In 1906 Erich, after reviewing the early work of Clementz, expressed the belief that the cartilage in mixed tumors of the salivary glands resulted from mucoid degeneration of the epithelial cells. Although the matter has never been entirely settled, there has been a strong tendency on the part of most writers in recent years to regard these neoplasms as essentially epithelial in nature; the connective tissue and cartilaginous portions, according to these authors, are the result of activity on the part of the epithelial cells. This tendency is supported by the following statement in the latest edition of Ewing's[13] classic work on neoplastic diseases:

> The long controversy over the origin of mixed tumors of the salivary glands reviewed in previous editions of this work and ending in the demonstration of their essential epithelial nature is now only of historical interest. Yet the proof of their epithelial nature has by no means completed the discussion of their histogenesis.

Many workers feel that the numerous morphologic studies undertaken have not furnished adequate proof that these tumors are purely epithelial in origin, and recently Hempelmann and Womack[14] have resorted to histochemical methods in an attempt to determine the true character of the various constituents. They were able to demonstrate both mesenchymal and epithelial mucus, and their findings furnish strong support for the concept that both ectoderm and mesoderm participate in the development of mixed tumors of the salivary glands.

It is the opinion of the majority that histogenetically these tumors are associated with a failure of normal development. Utilizing the "organizer" concept of Speeman, Hempelmann and Womack[14] suggested that the unusual appearance of the interstitial tissues may result from the influence of functionally inadequate ectoderm on mesodermal cells; it is recognized that "functional inadequacy on the part of one tissue at any phase during development may result in structural changes in all tissues concerned subsequently." Undifferentiated ectoderm or ectodermal primordia would therefore exert

12. No attempt is made in this paper to cover thoroughly the historical aspects of this problem. The reader is referred instead to the papers of others, who have covered the subject adequately (Ewing,[13] McFarland, J.: Am. J. M. Sc. **172**:804, 1926).

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14. Hempelmann, L. H., Jr., and Womack, N. A.: Ann. Surg. **116**:34, 1942.

a provocative action on the surrounding mesoderm, resulting in the formation of cartilage, bone and other heterotopic interstitial tissues.

It is probable that the same embryologic forces which operate in the development of one organ of ectodermal origin act in all of them. This contention is supported by the similar response of the mesenchyme to epithelial activity in the various organs which are derived from the ectoderm. The behavior of the mesenchymal tissues under conditions of growth and neoplasia may also be due solely or in part to the "organizer" influence. There is considerable evidence that this factor is retained to a certain degree by normal adult epithelium [15] and also by the tissues of tumors.[16]

SUMMARY

The 4 mixed tumors of the skin reported were located on the breast, the nose, the sternum and the auricle. Those arising within the skin of the breast and the sternum represent, so far as I know, the first reported neoplasms of this type located on the trunk.

The tumors situated within the skin of the breast and the nose were similar morphologically to the most common variety of mixed tumor, found in association with the salivary glands, and also to the rarer mixed tumors of the breast. The tumor which arose from the skin of the sternum presented certain structural features similar to those of the neoplasms located in the skin of the breast and the nose but appeared to represent a further stage in differentiation of both ectoderm and mesoderm. The tumor which arose from the auricle presented a morphologic appearance identical with that of another fairly common type of tumor ordinarily seen in the salivary glands, cylindroma. It is suggested, therefore, that mixed tumors of the skin, the salivary glands and the breast are similar morphologically, and further, that a given tumor may contain tissues representing all of the possibilities of ectodermal differentiation.

Mixed tumors of the salivary glands have been studied extensively from the standpoint of histogenesis. The data from those studies, together with the data furnished by morphologic studies of the mixed tumors of salivary gland type arising elsewhere in the body, suggest that mixed tumors of the skin, the salivary glands and the breast arise from abnormal epithelial cells which have similar developmental potentialities and which exert a provocative action on the mesoderm, resulting in the formation of heterotopic tissues of various types. The distribution of these tumors suggests further a direct relationship between the frequency of their occurrence in a given portion of the body and the complexity of the developmental process in that region.

The foregoing observations suggest, therefore, that mixed tumors arising in the skin, the breast and the salivary glands are histogenetically similar.

15. Huggins, C. B.: Arch. Surg. **22**:377, 1931.

16. Schmidt, I.: Virchows Arch. f. path. Anat. **291**: 491, 1933. Womack, N. A., and Graham, E. A.: Arch. Path. **26**:165, 1938.

General Reviews

RH BLOOD FACTORS: AN ORIENTATION REVIEW

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Progress in the field of blood groups has been extremely rapid in the past few years. As a result, the subject has become complicated and definitely more difficult to understand fully. The new developments, however, are not beyond the grasp of any educated person if he devotes to the study of them a little patience, if he understands the fundamental principles of genetics and if he knows a little about the antibody response which organisms may make to a foreign antigen. One of the most striking and important of the new developments is the work on the Rh factor, which will be reviewed here.

DISCOVERY OF DIFFERENTIATION OF BLOOD

Landsteiner's [1] discovery of the individual differences in human blood which underlie the four classic groups O, A, B and AB explained the severe or fatal reactions which often had resulted from transfusions attempted up to that time. It was natural that the idea of other possible agglutinogens in human blood should be entertained by workers attracted to this new field. Nevertheless it was again Landsteiner, working with Levine,[2] who first studied and named three of these additional properties (M, N and P). Other experimenters had perhaps had in their hands reagents for detecting such differences, but lack of time or interest, or insufficient serologic experience, had resulted in their not pursuing the subject.

As interest in the subject of blood grouping expanded, and the forensic usefulness of the M and N factors became known, attempts were made to identify other blood differences, and various workers reported new factors, more or less well characterized, which were designated by letters such as G, H. X, Q and E.[3] Some of these at least were probably related to the factor P. In most of the instances, continuity of reagents has been lost, and one will probably never know any more about the factor than may be learned from the author's original description. Other workers, such as Hooker and Anderson,[4] Zacho,[5] Culbertson and Ratcliffe,[6] Neter [7] and Levine and Stetson,[8] observed, but did not name, agglutinins and agglutinogens which seemed to fall outside the A-B and M-N schemes. As early as 1921 Unger [9] observed that weak agglutination might occasionally result on cross matching bloods of persons of the same blood group, and noticed that transfusion in such cases caused sharp reactions. The case of Levine and Stetson was particularly well described, and (to anticipate) later tests showed it to depend on the Rh factor.

DISCOVERY OF RH

In 1940 Landsteiner and Wiener,[10] using the serum of a rabbit immunized with erythrocytes from a rhesus monkey, demonstrated an agglutinin which agglutinated the red blood cells of about 85 per cent of the white population of New York, irrespective of the blood group. They designated the new agglutinogen, in conformity with the origin of the serum, as Rh. The technic of testing for the new factor was difficult, the best available serums were weak, and had it not been for a remarkable series of discoveries which followed in the next few months, the Rh factor might have aroused no more interest than its practically stillborn brethren, G, H, X, Q and E. Fully to under-

From the Boston University School of Medicine.

1. Landsteiner, K.: Wien. klin. Wchnschr. **14**:1132, 1901.

2. (*a*) Landsteiner, K., and Levine, P.: J. Exper. Med. **47**:757, 1928. (*b*) Landsteiner, K.; Strutton, W. R., and Chase, M. W.: J. Immunol. **27**:469, 1934.

3. Schiff, F.: Acta Soc. med. fenn. duodecim (Ser. A, art. 8) **15**:1, 1932; Naturwissenschaften **20**:658, 1932. Andresen, P. H.: Ztschr. f. Immunitätsforsch. u. exper. Therap. **85**:227, 1935. Furuhata, T., and Imamura: Jap. J. Genetics **12**:50, 1936; cited by Wiener.[11b] Sugishita: Juzenkai-Zasshi **40**:3367, 1935; cited by Schiff, F., and Boyd, W. C.: Blood Grouping Technic, New York, Interscience Publishers, Inc., 1942.

4. Hooker, S. B., and Anderson, L. M.: J. Immunol. **6**:419, 1921.

5. Zacho, A.: Hospitalstid. **78**:225, 1935; Ztschr. f. Rassenphysiol. **8**:1, 1936.

6. Culbertson, C. G., and Ratcliffe, A. W.: Am. J. M. Sc. **192**:471, 1936.

7. Neter, E.: J. Immunol. **30**:255, 1936.

8. Levine, P., and Stetson, R. E.: J. A. M. A. **113**: 126, 1939.

9. Unger, L. J.: J. A. M. A. **76**:9, 1921.

10. Landsteiner, K., and Wiener, A. S.: Proc. Soc. Exper. Biol. & Med. **43**:223, 1940.

stand the explosive outbreak of interest which greeted the new blood factor, one must again go back a little into the history of blood grouping.

"HETEROSPECIFIC PREGNANCY"

After the blood groups were discovered, workers who were aware that safe routine transfusions were possible only when donor and recipient were of compatible groups began to ask themselves whether blood group incompatibility between mother and fetus might not play a role in the intrauterine and neonatal deaths which are so common. Flexner [11] suggested that agglutination of the red blood corpuscles was the precipitating cause of the periportal necrosis of eclampsia. In 1905 Dienst [12] suggested that fetal red blood cells can enter the maternal circulation. McQuarrie [13] in 1923 collected data which he thought suggestive of the existence of some relationship between the incompatibility of maternal and fetal blood, on the one hand, and the development of eclampsia or preeclamptic toxemia, on the other. The concept of "heterospecific pregnancy," i.e, a pregnancy in which the blood group of the fetus is not compatible with that of the mother, was invented (Hirzfeld [14]) and enjoyed some vogue, especially after it was shown that the A and B antigens develop early in fetal life [15] and after observations of increase in the strength of isoagglutinins in the mother's blood when these were incompatible with an agglutinogen in the fetal blood.[16] In view of the known fact that antibodies can pass through the human placenta [17] it seemed that the concept might be an important one. More careful studies, however, did not support this idea.

In particular, Koller,[18] in a contribution which does not seem to be mentioned in any of the recent papers on the effects of incompatibility of the blood groups of mother and child, analyzed statistically the results of studies of the A, B, O groups of many families, including several thousand children, and concluded that the hypothesis of a detrimental effect on the child of A, B, O incompatibility with the mother was rendered by his results very improbable, though it was not completely disproved. (Modern work on the whole substantiates this conclusion, although suggesting that in some cases A, B, O incompatibility may play a certain role in the complications of pregnancy.)

Therefore the idea was generally abandoned, and when Diamond, Blackfan and Baty [19] suggested in 1932 that universal edema of the fetus, icterus gravis neonatorum and anemia of the newborn were all manifestations of the same disease and could conveniently be grouped together and known as "erythroblastosis fetalis," no thought of a possible serologic origin of the condition apparently was in their minds. Parsons, Hawksley and Gittins [20] concluded in 1933 that some hemolytic process was at work, but they were unable to identify it. Darrow [21] reviewed the pathology of the disease in 1938, emphasized its familial occurrence and suggested that "the placenta seems to be the means of transmission of the destructive influence from mother to fetus." She expressed the opinion that isoimmunization was the basis of the disease but also expressed the belief that the mother became immunized to fetal hemoglobin, which she had found was serologically different from adult hemoglobin. The antihemoglobin isoantibodies of the mother were supposed to be responsible for the destruction of the fetal blood.

ISOIMMUNIZATION

The term "antigen" is used in immunology in two senses; in the broader sense in which I have just been using it, the term designates a substance which reacts with an antibody; in the strict sense it designates a substance which when introduced parenterally into an animal causes the organism to produce antibodies corresponding to and reacting with the antigen. Are the blood group substances of which I have been speaking antigens in this sense? It seems that they are, for when red cells (and sometimes other materials) containing them are injected into rabbits, antibodies specific for the group antigens often appear. Other antibodies which act on all human cells in general may be produced at the same time but can be removed by absorption with cells lacking the specific blood

11. Flexner, S.: Univ. Pennsylvania M. Bull. **15**:324, 1902. Ottenberg, R.: J. A. M. A. **81**:295, 1923.
12. Dienst, A.: Zentralbl. f. Gynäk. **29**:353 and 651, 1905.
13. McQuarrie, I.: Bull. Johns Hopkins Hosp. **34**:51, 1923.
14. Hirszfeld, L.: Konstitutionsserologie und Blutgruppenforschung, Berlin, Julius Springer, 1928.
15. Kemp, T.: Acta path. et microbiol. Scandinav. **7**:146, 1930. Moureau, P.: Rev. belge d. sc. med. **7**:177 and 540, 1935. Semzova and Terechova: Centralbl. f. Blutgruppenforsch. **2**:130, 1929; Klin. Wchnschr. **8**:206, 1929; cited by Wiener.[71b]
16. (*a*) Jonsson, B.: Acta path. et microbiol. Scandinav. **13**:424, 1936. (*b*) Dienst.[12]
17. Sherman, W. B.; Hampton, S. F., and Cooke, R. A.: J. Exper. Med. **72**:611, 1940. Wiener, A. S., and Silverman, I. J.: ibid. **71**:21, 1940.
18. Koller, S.: Ztschr. f. Rassenphysiol. **3**:1, 1931.
19. Diamond, L. K.; Blackfan, K. D., and Baty, J. M.: J. Pediat. **1**:269, 1932.
20. Parsons, L. G.; Hawksley, J. C., and Gittins, R.: Arch. Dis. Childhood **8**:159, 1933.
21. Darrow, R. R.: Arch. Path. **25**:378, 1938.

group substance in question. If red cells containing the M (or N) antigen are injected into rabbits, agglutinins specific for the M (or N) agglutinogen (plus other agglutinins for human erythrocytes in general) may be formed. In this sense one is justified in speaking of M and N as antigens.

It has been known for a long time [22] that the blood of some animals may contain antigens which when the blood is injected into another individual of the same species whose blood lacks these particular antigens will cause the production of antibodies. Such antigens are called isoantigens. Since 1900 isoimmunization has been accomplished with various other species, such as rabbits [23] and mice.[24] More recently, following work of Biancalana and Teneff [25b] and Aubert, Boorman and Dodd,[25a] it has been shown by Witebsky, Klendshoj and McNeil [26] and Wiener, Sonn and Belkin [27] that a single intravenous injection of a very small dose of purified, even apparently protein-free A or B substance will usually greatly increase the titer and avidity of the anti-A or anti-B agglutinins (or both if present) of a normal person.

Therefore, another natural outgrowth of the new knowledge of blood incompatibility was the concept that transfusions of blood containing a factor not present in the recipient's blood, even though no natural isoagglutinin was present at the outset, might result in the formation of an immune isoagglutinin or produce some other form of sensitization, so that later transfusions of blood containing this factor could eventually be harmful. This was specifically suggested in the case of the M, N series by Lattes,[28] and the warning was reiterated by numerous others. As fate would have it, the M and N factors, though antigenic for rabbits, seem seldom to be isoantigenic in man, so the difficulty which was feared did not immediately arise. In only a few of all the bloods that have been examined have human anti-M [29] agglutinins been described. It is for this reason (and because agglutinins for M and N are normally lacking in human blood) that it is not important to take account of the M, N types in performing blood transfusions. Wiener and Unger have observed 3 cases of anti-P isoimmunity to date.

Nevertheless, accidents and untoward results [30] continued to occur in transfusions much more frequently than many clinicians were willing to admit. Some of the reactions, but probably a minority, may have been due to the use of a "dangerous universal donor" [31] whose anti-A and anti-B agglutinins were unusually potent and when transfused caused destruction of the recipient's erythrocytes or other undesirable effects. Davidsohn [32] argued, principally from circumstantial evidence, that some transfusion reactions resulted from subgroup incompatibilities (e. g., A_2 vs. A_1 or A_1B vs. A_2B and vice versa), but direct support of this contention was first produced by Wiener.[33] Zacho [6] described a transfusion incompatibility (perhaps due to one of the Rh factors), and other observers noted similar occurrences.

Although the reports apparently made little impression, a number of workers observed that occasionally even in cases in which a husband and his wife were of the same blood group and cross matching carried out in the usual way demonstrated no incompatibility, the blood of the husband when transfused into the postpartum wife caused a severe reaction. As an example the case of Culbertson and Ratcliffe,[6] reported in 1936, may be cited:

Mrs. C. was admitted to the maternity ward of the hospital and given a trial labor at the term of a normal pregnancy. Failing to make progress she was delivered

22. Ehrlich, P., and Morgenroth, J.: Berl. klin. Wchnschr. **37**:453, 1900. Todd, C., and White, R. G.: Proc. Roy. Soc., London, s.B **82**:416, 1910; J. Hyg. **10**:185, 1910.

23. Levine, P., and Landsteiner, K.: J. Immunol. **17**: 559, 1929; **21**:513, 1931. Marcussen, P. V.: Ztschr. f. Immunitätsforsch. u. exper. Therap. **89**:453, 1936. Fischer, W., and Klinkhart, G.: Arb. a. d. Staats.-Inst. f. exper. Therap., 1930, no. 23, p. 65. Boyd, W. C., and Feldman, D.: J. Immunol. **27**:547, 1934.

24. Gorer, P. A.: Brit. J. Exper. Path. **17**:42, 1936; **18**:31, 1937.

25. (*a*) Aubert, E. F.; Boorman, K. E., and Dodd, B. E.: J. Path. & Bact. **54**:89, 1942. (*b*) Biancalana, L., and Teneff, S.: Soc. internaz. di microbiol., Boll. d. sez. ital. **3**:613, 1931. (*c*) Krah, E.: Ztschr. f. Immunitätsforsch. u. exper. Therap. **101**:133, 1942. (*d*) Mollison, P. L., and Young, I. M.: Lancet **2**:635, 1941.

26. Witebsky, E.; Klendshoj, N. C., and McNeil, C.: Proc. Soc. Exper. Biol. & Med. **55**:167, 1944.

27. Wiener, A. S.; Sonn, E. B., and Belkin, R. B.: J. Exper. Med. **79**:235, 1944.

28. Lattes, L.: The Individuality of the Blood in Biology and in Clinical and Forensic Medicine, translated by L. W. H. Bertie, London, Oxford University Press, 1932.

29. (*a*) Paterson, J. L. L. H.; Race, R. R., and Taylor, G. L.: Brit. M. J. **2**:37, 1942. (*b*) Wiener, A. S., and Forer, S.: Proc. Soc. Exper. Biol. & Med. **47**:215, 1941. (*c*) Taylor, G. L., and Race, R. R.: M. Press **211**:86 and 104, 1944.

30. DeGowin, E. L., and Baldridge, C. W.: Am. J. M. Sc. **188**:555, 1934. Mandelbaum, H.: Ann. Int. Med. **12**:1699, 1939.

31. DeGowin, E. L.: J. A. M. A. **108**:296, 1937. Muller, M., and Balgairies, E.: Compt. rend. Soc. de biol. **121**:1447, 1936. Wiener, A. S., and Moloney, W. C.: Am. J. Clin. Path. **13**:74, 1943. Aubert, E. F.; Boorman, K. E.; Dodd, B. E. and Loutit, J. F.: Brit. M. J. **1**:659, 1942. Levine, P., and Mabee, J.: J. Immunol. **8**:425, 1923.

32. Davidsohn, I.: J. A. M. A. **112**:713, 1939.

33. Wiener, A. S.: J. Immunol. **41**:181, 1941.

by Cesarean section. Considerable blood was lost. Two days later she was given a blood transfusion from her husband who was found to be of the same group (O). Direct cross-matching of the bloods in hanging-drop preparations with 20 minutes incubation at 37° C. showed no evidence of agglutination under the microscope.

At the completion of the transfusion the patient had a chill and her temperature rose to 105° F. This was followed by hemoglobinuria and oliguria for several days, during which time the blood T. N. P. N. [total nonprotein nitrogen] gradually rose to 235 mg. per 100 cc. The patient's condition was regarded as critical by the clinicians who saw her. She was given symptomatic treatment, the details of which are outside the scope of this paper. After a very anxious period she gradually recovered; at the end of 7 weeks she was symptom-free and had a normal T. N. P. N. and urine. The patient had never been transfused prior to this time. She gave no history of allergic manifestations.

Levine and Stetson [8] reported a transfusion reaction in a woman (group O) just delivered of a macerated fetus, who experienced a severe chill and pains in the legs and the head following a transfusion of her husband's blood (group O). They found in her serum moderately active atypical agglutinins which agglutinated about 80 per cent of the group O bloods tested. Subsequent transfusions from 6 group O donors selected so as to be compatible on cross matching (i. e., lacking the unknown agglutinogen present in the husband's and many other bloods) were successful. Levine and Stetson pointed out that in instances of isoimmunization in animals the isoagglutinin generally serves as a reagent to detect a dominant hereditary blood factor.

ISOIMMUNIZATION OF MOTHER BY FETUS

To explain the occurrence of the atypical agglutinin in the maternal circulation in their case, Levine and Stetson [8] suggested the important idea that foreign antigen from the fetus (supposed to have inherited the factor from the husband) had passed through the placenta and, acting as an isoantigen, immunized the mother.

This suggestion was in direct line with Jonsson's observations [16a] of increase of isoagglutinin titer, already referred to, and was shown by Levine to be the key to the etiology of hemolytic disease of the newborn.

The observations of Wiener and Peters,[34] by demonstrating that the new Rh factor when transfused into the blood of recipients whose red cells did not contain it could act as an isoantigen, served to suggest that this idea was correct.

The ground was now all prepared, and the seed planted by Landsteiner and Wiener's discovery and characterization of the Rh factor produced a rapid and luxuriant growth of fruitful investigations. Wiener and Peters reported three hemolytic transfusion reactions due to Rh incompatibility.[34] They suspected [35] that the transfusion reactions observed by Levine and Stetson and other previous workers were due to Rh incompatibility, and later when anti-Rh serums became available to Levine and Katzin,[36] the latter workers were able to show that such was the case: The red cells of the patient lacked Rh, those of the husband contained Rh, the atypical agglutinin was anti-Rh in specificity, and the red cells of all the mother's compatible donors were Rh free.

CHARACTERISTICS OF THE RH FACTOR

Mollison [37] pointed out that the reason anti-Rh agglutinins in human serum had passed almost completely unnoticed for so many years (and their importance when observed had not been recognized) was undoubtedly largely that poor reactions were given by these agglutinins in vitro.

Although Wiener was at first inclined to believe that the greater difficulty of testing for Rh was due to the Rh antigen being located below the surface of the red cell, Wiener, Sonn and Belkin have more recently suggested that the reason is rather that far fewer Rh haptens (combining groups) occur on each erythrocyte than in the case of A and B.

It was at first thought that the Rh agglutinogen was confined to the erythrocytes,[38] but Boorman and Dodd claimed that the Rh factor (in non-water-soluble form) occurs in other tissues, as do A and B. If this is so, only the relatively small amount of water-soluble Rh in the tissue fluids could play much role in protecting the infant's erythrocytes against the action of the anti-Rh antibodies diffusing through from the mother's circulation. This may help account for the greater role of Rh in hemolytic disease of the newborn.

Rh antibodies have been demonstrated in breast milk by Witebsky and co-workers,[39] by Langley and Stratton,[40] by Fisk and Foord [41]

34. Wiener, A. S., and Peters, H. R.: Ann. Int. Med. **13**:2306, 1940.

35. Levine, P.: J. Pediat. **23**:656, 1943.

36. (*a*) Levine, P., and Katzin, E. M.: Proc. Soc. Exper. Biol. & Med. **45**:343, 1940. (*b*) Levine, P.: Arch. Path. **37**:83, 1944.

37. Mollison, P. L.: Post-Grad. M. J. **20**:17, 1944.

38. (*a*) Levine, P.: Am. J. Obst. & Gynec. **42**:165, 1941. (*b*) Wiener and Forer.[29b]

39. Witebsky, E.; Anderson, G. W., and Heide, A.: Proc. Soc. Exper. Biol. & Med. **49**:179, 1942. Witebsky, E., and Heide, A.: ibid. **52**:280, 1943.

40. Langley, F. A., and Stratton, F.: Lancet **1**:145, 1944.

41. Fisk, R. T., and Foord, A. G.: Am. J. Clin. Path. **14**:431, 1944.

and by Wiener.[42] This observation may possibly help account for the persistence and the exacerbation of hemolytic disease of the newborn after birth.

The first human anti-Rh serums studied by Wiener [34] were found to be more active in the cold than at room or higher temperatures (Dockeray and Sachs [43] also recently described a "cold" anti-Rh serum), but the great majority of serums were found by Levine and associates [44] and by Wiener [45] to contain "warm" agglutinins (i. e., agglutinins more active at 37C. than at room or lower temperatures); so in making Rh tests it seems generally advisable to incubate the cell-serum mixture at 37 C. for an hour or longer before making a reading. Landsteiner and Wiener [46] recommended that the reactions of the original guinea pig anti-Rh serum be read by inspection of cell sediment after incubation. With good human anti-Rh serums macroscopic, or at least microscopic, agglutination is visible after incubation, mild centrifugation and gentle shaking. Diamond and Abelson [47] described a slide test which seems to be extremely reliable.

Some human serums seem to contain a "blocking antibody" [48] for anti-Rh agglutinin, which prevents the in vitro action of the agglutinin but does not itself agglutinate. Wiener expressed the belief that the presence of some of this antibody explains the "optimal proportions" effect found by Taylor and co-workers,[49] and it may also explain the lack of reactivity of certain pooled and processed anti-Rh serums. The slide test of Diamond and Abelson is positive even with anti-Rh serum containing the blocking antibody.

If the human anti-Rh serum comes from a person not of group AB, and it is desired to test other than group O cells, it is necessary to neutralize any anti-A or anti-B agglutinin present before using the serum for Rh tests. Wiener and Forer [29b] used human saliva from "secretors" [50] for this purpose; later Levine and associates,[51] Wiener and others found that purified A and B substances when available were useful for this purpose.

RELATION OF RH TO TRANSFUSION REACTIONS

The 3 transfusion reactions due to Rh incompatibility observed by Wiener and Peters [34] have already been mentioned. In 1941 Wiener [45] reported 10 further cases in which transfusion of Rh-containing blood produced an untoward reaction.

As indicated in previous and subsequent reports,[52] these reactions fell into two natural subdivisions: (1) those in patients receiving repeated transfusions in whom the source of isoimmunization was the blood given in the previous transfusions, and (2) those in patients who had never previously been treated by transfusion. The latter patients were always women who had become sensitized by pregnancy. Wiener noted that some of the patients who had the most severe reactions had no demonstrable anti-Rh agglutinins in their serums. He now believes that the failure to demonstrate the antibodies in their serums can be explained by the presence of "blocking antibodies," mentioned in an earlier paragraph.

ROLE OF RH IN ERYTHROBLASTOSIS

Following up the suggestion of Levine and Stetson,[8] Levine, Burnham, Katzin and Vogel [51] considered more fully the role of isoimmunization in the genesis of erythroblastosis fetalis. They considered that isoimmunization of a mother to Rh antigen contained in her fetus, with subsequent passage of the immune anti-Rh agglutinin back across the placenta, was the cause of erythroblastosis in the great majority of cases. As statistical evidence they pointed out that whereas only 15 per cent of a random sample of the population gave red blood cells that were negative when tested with anti-Rh serum, of the 153 mothers whose infants were affected with erythroblastosis fetalis, 141 (92 per cent) gave negative red cells. Moreover, of 76 infants and 89 husbands tested in this group, *all* were shown by positive tests to have Rh in their red cells. In 70 cases the mother's serum was tested for anti-Rh agglutinins within two months after delivery; the antibodies were found in 33 of these. Confirmatory observations have been

42. Wiener, A. S.: Am. J. Clin. Path. **14**:52, 1944.

43. Dockeray, G. C., and Sachs, H.: J. Immunol. **48**:241, 1944.

44. Levine, P.; Katzin, E. M., and Burnham, L.: Proc. Soc. Exper. Biol. & Med. **45**:346, 1940. Levine, P.: Am. J. Clin. Path. **11**:898, 1941.

45. Wiener, A. S.: Arch. Path. **32**:227, 1941.

46. Landsteiner, K., and Wiener, A. S.: J. Exper. Med. **74**:309, 1941.

47. Diamond, K., and Abelson: J. Clin. Investigation **24**:122, 1945.

48. Race, R. R.: Nature, London **153**:771, 1944. Wiener, A. S.: Proc. Soc. Exper. Biol. & Med. **56**: 173, 1944.

49. Taylor, G. L.; Race, R. R.; Prior, A. M., and Ikin, E. W.: Brit. M. J. **2**:572, 1942.

50. Schiff, F., and Sasaki, H.: Klin. Wchnschr. **11**: 1426, 1932.

51. Levine, P.; Burnham, L.; Katzin, E. M., and Vogel, P.: Am. J. Obst. & Gynec. **42**:925, 1941.

52. (*a*) Vogel, P.; Rosenthal, N., and Levine, P.: Am. J. Clin. Path. **13**:1, 1943. (*b*) Broman, B.: Acta pædiat. (supp. 2) **31**:1, 1944. (*c*) Ayer, G. D., Jr., and Kammer, W. F.: Arch. Int. Med. **73**:199, 1944.

published by Wiener,[53] Boorman, Dodd and Mollison [54] and others (table 1).

Levine [55] has suggested that serologic incompatibility (perhaps in regard to other blood factors also) may sometimes be responsible for spontaneous abortions and stillbirths. He [56] also mentioned that several workers had reported a higher than normal incidence of congenital malformations in erythroblastotic infants. Cook [57] has suggested that Rh incompatibility may explain certain cases of feeblemindedness.

TABLE 1.—*Statistical Relationship of Erythroblastosis Fetalis to Presence or Absence of Rh in Mother's Red Cells*

Authors	Number of Mothers Whose Red Cells Contained Rh	Number of Mothers Whose Red Cells Did Not Contain Rh
Boorman, Dodd and Mollison [54]	2	46
Potter, E. L.; Davidsohn, I., and Crunden, A. B.: Am. J. Obst. & Gynec. 45:254, 1943	6	54
Brewer, H. F.: St. Barth. Hosp. J., War Ed. 4:48, 1943	0	5
Wiener [53]	6	37
Race and associates [76c]	6	44
Diamond, L. K.: Personal communication to the author	10	65
Total number	30	251
Per cent	10.3	89.7

It was clear from the start that the combination Rh-free maternal cells and Rh-containing fetal cells, which occurs in about 9.5 per cent of all cases of pregnancy in England and North America, does not automatically mean erythroblastosis, for the highest estimate of the incidence of this disease is only about 1 in 200 or 1 in 400 births.[58] As Mollison [37] pointed out, therefore, only about once in forty times does the potentially dangerous combination actually lead to morbidity. Evidently certain other conditions must also be fulfilled. What they all are is not known, but one essential seems to be the passage of Rh-containing fetal cells (or possibly just Rh antigen?) through the placenta into the maternal circulation, with resulting immunization (unless this has already happened in a previous pregnancy; Javert [59] pointed out in 1942 that multiparity was a factor in 92 per cent of the mothers), and diffusion of the resulting anti-Rh substance back into the fetal circulation, where it causes the destruction of erythrocytes, resulting in erythroblastosis. Why this does not always accur, workers in this field do not yet know. It has been suggested that a hereditary difference in placental permeability might be responsible.[60] This is perhaps supported by the observation that when a child with erythroblastosis has been born later children are likely to be born with it unless they are Rh free. Wiener and Wexler [61] expressed the belief that the most important factor is the mother's presensitization and her capacity to be easily sensitized. Data on the persistence of anti-Rh agglutinins are shown in table 2, modified from Levine. Levine [36b] has also shown that very small doses of human cells can cause distinct production of species-specific agglutinin, in rabbits at least.

Contrariwise, the fact that a mother's red cells have given a positive reaction with an anti-Rh serum does not mean that she cannot have an erythroblastotic infant, although the chances of it are much less.[58] That this can happen is at least partly explained by differences in subtypes of Rh, which will be discussed in the following section.

Because of limitations of space, and because the subject will be found thoroughly discussed in some of the articles referred to here, I shall not go further into the question of the clinical nature of erythroblastosis fetalis (hemolytic disease of the fetus and the newborn).

TABLE 2.—*Persistence of Anti-Rh Agglutinin in Two Hundred and Six Mothers Whose Red Cells Showed No Rh but Whose Infants Had Rh in Their Cells*

Interval After Last Delivery of an Affected Infant	Mothers in Whose Blood Rh Agglutinin Was Present	Mothers in Whose Blood Rh Agglutinin Was Not Shown
2 months post partum	58	44
2 months to 1 year post partum	15	29
1 year or longer post partum	7	43
During next pregnancy	2	5
No data	0	3
Total	82	124

SUBDIVISIONS OF THE RH TYPE

Soon after the importance of the Rh factor in erythroblastosis fetalis and in transfusion reactions was established, it was observed that human

53. Wiener, A. S.: Science **96**:407, 1942.

54. Boorman, K. E.; Dodd, B. F., and Mollison, P. L.: Brit. M. J. **2**:535 and 569, 1942; J. Obst. & Gynaec. Brit. Emp. **51**:1, 1943.

55. Levine, P.: J. Hered. **34**:71, 1943.

56. Levine, P.: New York State J. Med. **42**:1928, 1942.

57. Cook, R.: J. Hered. **35**:133, 1944.

58. Javert, C. T.: Am. J. Obst. & Gynec. **43**:921, 1942.

59. Javert, C. T.: Surg., Gynec. & Obst. **74**:1, 1942; footnote 58.

60. Haldane, J. B. S.: Ann. Eugenics **11**:333, 1942.

61. Wiener, A. S., and Wexler, I. B.: Am. J. Clin. Path. **13**:393, 1943.

serums containing anti-Rh agglutinins did not exhibit identical specificities.[62] For a time the three principal types of such serums were designated by the characteristic percentages [51] of positive reactions obtained with them on bloods of the white population of New York and vicinity, that is, as 87 per cent, 85 per cent and 73 per cent, respectively. Levine [63] showed that the 87 per cent serum contained more than one antibody, and, in fact, it is now known that it contains the two agglutinins found separately in the 85 and 73 per cent serums. At first it was thought that Rh could be separated simply into Rh_1 and Rh_2, as in the case of A,[64] but later work [65] has shown that the subject is actually more complex than it first appeared. Therefore, the earlier nomenclatures proposed for these agglutinins and their corresponding agglutinogens have been somewhat modified in the light of later results.[27] I shall accordingtly present only the nomenclature devised by Wiener,[67] which seems likely to be accepted by other workers in the field. It has the advantages of not being very different from the nomenclatures first proposed and of having certain features which make it fairly easy to remember.

Before an attempt is made to explain fully the subtypes of the Rh factor, a brief account of the subgroups of the classic four blood groups, particularly a short sketch of the basis of their inheritance, will be desirable. There are certain observations which suggest that the chief obstacle to an understanding of the subdivisions of the Rh factor, particularly on the part of clinicians, is lack of familiarity with the genetics of the blood groups. Fortunately, this is a deficiency which is easily remedied, and it does not seem at all impossible to give in the space available here a sketch of the subject which will be sufficient so far as the immediate problem of understanding the newer work in blood groups is concerned. Those already familiar with genetic science may skip over the next few paragraphs, passing directly to those dealing with blood groups and types, or to the Rh factor.

62. Levine and others.[51] Wiener.[45]

63. Levine, P.: Proc. New York State A. Pub. Health Lab. **22**:43, 1942; footnote 56.

64. Wiener, A. S.: Am. J. Clin. Path. **12**:302, 1942.

65. (*a*) Wiener, A. S., and Landsteiner, K.: Proc. Soc. Exper. Biol. & Med. **53**:167, 1943. (*b*) Wiener, A. S.: ibid. **54**:316, 1943. (*c*) Kracke, R. R., and Platt, W. R.: Ann. Int. Med. **20**:559, 1944. (*d*) Race, R. R., and Taylor, G. L.: Nature, London **152**:300, 1943. (*e*) Race, R. R.; Taylor, G. L.; Boorman, K. E., and Dodd, B. E.: ibid. **152**:563, 1943.

66. Footnote deleted by the author.

67. Wiener, A. S.: Science **99**:532, 1944.

GENES

The inherited characteristics of animals and plants are determined by submicroscopic units called genes. These genes are arranged serial fashion in rodlike structures which are visible under suitable conditions in the nuclei of reproductive (and other) cells. These rods are called chromosomes. All the genes in any one chromosome are (apart from occasional transitions such as "crossing over" and the like) transmitted together from parent to offspring, and the number of gene groups in heredity is observed to correspond to the number of chromosomes in the reproductive cells of that species. This number varies from species to species.

In the fertilized gamete and in the adult organism the cell nuclei are observed to contain twice the number of chromosomes found in a reproductive cell; in fact, they contain two of each sort of chromosome, one member of each pair from the father and one from the mother. The respective members of a pair are very much alike, sometimes identical, in the genes they contain, and in the order in which the genes are arranged, and any difference is a reflection of a difference between the genetic constitutions of the mother and the father. In general, however, this rule will hold: *For any particular gene, or for other genes which can substitute for it in inheritance, there will be available in the nucleus of the fertilized gamete just two loci, one at some point of a certain chromosome, and another at the corresponding point of the other member of the chromosome pair.*

As long as only one kind of gene is available to fill these loci, all fertilized gametes are alike in regard to this particular kind of gene, and all offspring of every mating will be identical in respect to the characters determined by it. The situation first becomes interesting when there is available more than one kind of gene which can occupy this particular locus on the chromosome. Then there is a chance for parents (and offspring) to differ in regard to this characteristic.

As the simplest case, I may begin with the inheritance of a serologic characteristic which is perhaps not as thoroughly understood as it might be, namely, the blood types that are determined by the factors M and N, discovered in 1928 by Landsteiner and Levine.[2a] At a certain locus of a certain chromosome there may occur a gene which has been designated as M. At the corresponding locus of the other chromosome which pairs with this, there may be found a gene which has been designated as N. The two kinds of chromosome in European ("white") populations

occur with nearly equal frequency. Since men and women do not choose their mates on the basis of their blood types, the random mating which results gives three sorts of combinations of chromosomes, a pair with M on both, a pair with N on both and a pair of which one bears M and the other N. Since I am not concerned at present with the other genes in the chromosomes, I may represent these simply by MM, NN and MN. So far as any one knows, the sole result of the gene M is to cause the production of the M antigen (recognizable by agglutination tests with properly absorbed immune rabbit serum) in the red cells of the person inheriting the gene. Since the gene N causes the production of a similar but distinct antigen, N, and since neither gene interferes very much with the action of the other member of the gene pair if it is also present, the result is to give a population divided into three blood types: those whose cells contain M, those whose cells contain N and those whose cells contain both M and N. The genetic constitution of a person is called his "genotype," and the resultant observable physical constitution is called his "phenotype." This will serve to explain table 3.

TABLE 3.—*Gene Series M, N*

Genotype	Phenotype
MM	M
MN	MN
NN	N

DOMINANCE

As has already been said, this is the simplest possible case. (As a matter of fact, there may exist two kinds of N gene,[68] but that may be ignored here.) A slightly more complicated case would occur if the presence of one gene prevented the development of the antigen ordinarily produced by the other. If this had been true in the M, N series, for example, the MM and MN genotypes might both turn out to be phenotypically just M. In such a case one gene is said to be dominant over the other, which is said to be recessive. However, dominance is by no means always observed, even if one scrutinizes all combinations of a gene series. It has been plausibly argued that dominance is a device developed in the course of evolution to minimize the bad effect of deleterious genes,[69] since deleterious genes are generally found to be recessive. It should be noted that the dominance of one gene over the other, in the sense of a repression of the effects of the second gene, lasts only so long as the genes are in association in the same organism. If in the next generation they again get separated, the gene which was temporarily eclipsed, if not again combined with a dominant gene, will again exert its effects. Furthermore, the frequency of the two genes in the population is not progressively affected by the mere fact that one is dominant or recessive.

In some cases the presence of a single gene seems to cause as strong a development of the character it influences as when it is present in double dose (i. e., when an identical gene is in the corresponding locus of the other member of the chromosome pair). In the case of M and N, a single gene, as in the MN genotype, does not cause quite as strong a development of the antigen as when two identical genes are present, so that the reaction of the MN type ("heterozygote") is weaker both with anti-M and with anti-N agglutinins than the reactions of the MM and NN types ("homozygotes"). Wiener and co-workers[70] have actually presented evidence which suggests that the activity of the gene N is somewhat more repressed in the heterozygote than is the gene M, so that one might almost speak of a partial dominance of M over N. Nevertheless, it is always perfectly easy to recognize the presence of antigen N in the MN type; so the degree of dominance is relatively small.

ALLELOMORPHS

Another complication which may occur is the availability of more than two genes to fill the two loci in the chromosome pair in which they occur. In such a case it is obvious that no one person may have more than two of the available genes, and a number of different genotypes and phenotypes may occur in different members of the population. This is what happens in the case of the classic blood groups which were discovered in 1900 by Landsteiner.[1] According to the theory of their inheritance, worked out by Bernstein and now recognized as correct, these groups depend on the presence or the absence of three genes which are designated as O, A and B, all capable of occupying the same two loci on the chromosome pair which carries them (incidentally, a different pair from that carrying M and N). Both A and B are dominant over O; so the various combinations which result give

68. Crome, W.: Deutsche Ztschr. f. d. ges. gerichtl. Med. **24**:167, 1935. Friedenreich, V.: ibid. **25**:358, 1936.

69. Fisher, R. A.: The Genetical Theory of Natural Selection, London, Oxford University Press, 1930.

70. Wiener, A. S.; Zinsher, R., and Selkowe, J.: J. Immunol. **27**:431, 1934.

the genotypes and phenotypes shown in table 4. Genes forming members of a series all capable of occupying the same chromosome site are called allelomorphs (or alleles). In this case a single dose of A (or B) seems to be nearly or quite indistinguishable from a double dose; so dominance may be said to be complete.

TABLE 4.—*Gene Series O, A, B*

Genotype	Phenotype
OO	O
AO AA	A
BO BB	B
AB	AB

In European stocks, chromosomes carrying genes O, A and B, respectively, occur in about the proportion of 67, 26 and 7.

SUBGROUPS OF A

There is good reason to believe that the theory of inheritance of Bernstein, though correct as far as it goes, is in some respects a simplification. In the first place, there have been found to be two main sorts of antigen A, designated as A_1 and A_2.[71a] The former gives, for instance, stronger reactions with the ordinary anti-A serum. To explain this fact, and the data on the inheritance, Thomsen and others have proposed that the series of genes actually available for this locus consists of four genes, O, A_2, A_1 and B. A_1 is supposed to be dominant over A_2. Predictions based on such a theory agree well with the

TABLE 5.—*Gene Series O, A_2, A_1, B*

Genotype	Phenotype
OO	O
A_2O A_2A_2	A_2
A_2A_1 A_1O A_1A_1	A_1
BO BB	B
A_2B	A_2B
A_1B	A_1B

results on the whole. The possible genotypes and phenotypes are shown in table 5.

Although this accounts for the vast majority of the observations, there is still reason to think that it may not be the whole story. Friedenreich, Hirszfeld and others have suggested the presence of two other genes, A_3 and A_4, much weaker than A_2 even, and other workers have obtained some evidence for a possible division of the B gene into B_1 and B_2. Wiener[71b] expressed the belief that there is a gene $A_{1,2}$ determining an agglutinogen intermediate between A_1 and A_2. (Since the primary interest here is not in the A, B blood groups, the working out of all the possible combinations of the gene series O, A_4, A_3, A_2, A_1, B_2, B_1 is omitted.)

TABLE 6.—*The Landsteiner Blood Groups*

Earlier Nomenclature of		Present International Nomenclature	Agglutinogen in Cells	Agglutinin in Serum
Jansky	Moss			
I	IV	O	—	Anti-B and Anti-A
II	II	A	A	Anti-B
III	III	B	B	Anti-A
IV	I	AB	A + B	—

AGGLUTININS

I turn now to the question of the agglutinins by which these various antigens are detected. The M and N antigens are detected by the aid of absorbed serums from rabbits immunized with corpuscles containing the respective antigens. A and B can be similarly detected, but there is in addition, in this case, another source of agglutinin which is historically and even practically more important. This is normal human serum or plasma. As most readers know, the serum of each person contains anti-A or anti-B agglutinin in accordance with Landsteiner's rule, which states that agglutinin, and only that, will be found which can coexist physiologically with the antigen (agglutinogen) in the cells. This is shown in table 6.

This is all the information one needs about agglutinins if one ignores the subgroups A_2, A_1,

TABLE 7.—*Kinds of Natural Human Anti-A Serums*

Agglutinin	Found in Serum of	Reacts with Phenotype
Anti-A_2(O)	A_1B (rare) and A_1 (rarer) and B (rarest)*	A_2 and A_2B (very weak) (and group O) and perhaps weakly in heterozygous A_1 and B (?)
Anti-A_1	A_2B and A_2 (rare)	A_1 and A_1B
Anti-A_1 + Anti-A	B (usual)	All types of A and AB

* Wiener has observed a case (unpublished) of a group B mother with anti-O agglutinins.

etc. If they are considered, one finds that there can be present at least three fairly distinct varieties of anti-A agglutinin, although the propriety of calling one of them (the first shown in table 7) an anti-A agglutinin is a little doubtful, because it agglutinates also cells of group O. Table 7

71. (*a*) Landsteiner, L., and Levine, P.: J. Immunol. **17**:1, 1929. (*b*) Wiener, A. S.: Blood Groups and Transfusion, ed. 3, Springfield, Ill., Charles C Thomas, Publisher, 1943.

shows the agglutinins which are found naturally in human serums of various groups and which react with the various A antigens. The commonest type of anti-A serum, shown at the bottom of the table, is ordinary group B serum, which seems to contain two sorts of agglutinins, one, anti-A, which reacts both with A_1 and A_2, and another, anti-A_1, reacting predominantly with A_1. Anti-A_1 anti-B and anti-O agglutinins react best below 20 C. In the serum of certain persons, particularly those of the subgroups of AB, other anti-A agglutinins may be found. That found sometimes in the serum of group A_2B may perhaps be identical with the anti-A_1 in group B serum. The anti-A_2 found in the serums of certain persons of group A_1B reacts also with O, as has been mentioned; in this respect it behaves like an agglutinin which is found in the serum of certain cattle, which similarly agglutinates human bloods of groups O and A_2. The serum of cattle, however, may very rarely yield an agglutinin

TABLE 8.—*Gene Series O, A_2, A_1, B: Reactions of Phenotypes with Various Anti-A Agglutinins*

Phenotype	Reaction with Stated Agglutinin		
	Anti-A_1	Anti-A	Anti-A_2 (or Anti-O)
O	—	—	+
A_2	—	+	+
A_1	+	+	<± (weak ?)*
B	—	—	<± (weak ?)*
A_2B	—	+	+†
A_1B	+	+	—

* The response is irregular.
† The response is very weak and irregular.

really specific for O, i. e., one which does not agglutinate A_2.[72]

The reactions of the various subgroups (leaving out of account A_3, A_4, B_1, B_2 and other rarer possibilities) with the three kinds of anti-A agglutinins are shown in table 8.

A point to be noted is that one of the agglutinins may react with more than one variety of A, and, conversely, one variety of A may be agglutinated by more than one of the agglutinins.

ISOIMMUNIZATION

I have already shown that the factors A and B (and their subvarieties) seem to be isoantigenic in man. Table 9 presents the theoretic possibilities of antibody production in the case of each hypothetically possible combination among the six major groups and subgroups. It will be seen that they are surprisingly great. Of course, in many cases some antibody of that type would already be present, and also many of the indicated transfusions would never be performed except through an error. The chief importance of the table is to aid in understanding the observations on the Rh factor, to which I now turn.

72. Boyd, W. C.: Unpublished data.

THE RH GENES

The discovery of Rh has already been described. The first serums used enabled two new

TABLE 9.—*Theoretic Possibilities of Isoimmunization in O, A_2, A_1 and B*

Recipient	Donor Blood and Agglutinins Possibly Produced by It					
	O	A_2	A_1	B	A_2B	A_1B
O	—	Anti-A	Anti-A_1 (and anti-A ?)	Anti-B	Anti-A and anti-B	Anti-A, anti-A_1 and anti-B
A_2	—	—	Anti-A_1	Anti-B	Anti-B	Anti-A_1 and anti-B
A_1	Anti-A_2 (O)?	Anti-A_2	—	Anti-B	Anti-A_2 and anti-B	Anti-B
B	—	Anti-A and/or anti-A_2	Anti-A_1 (and anti-A)	—	Anti-A (and anti-A_2)	Anti-A_1 (and anti-A)
A_2B	—	—	Anti-A_1	—	—	Anti-A_1
A_1B	Anti-A_2 (O)*	Anti-A_2	—	—	Anti-A_2	—

* A case was reported by A. S. Wiener, B. H. Oremland, M. A. Hyman and A. A. Samwick (Am. J. Clin. Path. 11: 102, 1941).

types of human blood to be distinguished, that in which the red cells contained Rh and that in which the red cells were free from this factor, and it was postulated that the new gene series involved was Rh, rh, with Rh dominant over rh.[46] This led to the simple scheme shown in table 10, which showed a situation similar to the M,N series except that the first gene was completely dominant and no reagent for the other gene was then known. Evidence was obtained[73] which suggested that this new set of blood group genes was not on the same pair of chromosomes as either the M,N or the O,A,B series but on a different pair.

TABLE 10.—*Gene Series rh, Rh*

Genotype	Phenotype
rhrh	rh (Rh—)
rhRh	Rh (Rh+)
RhRh	

It was found that the Rh series resembled the O,A,B series and was different from the M,N series in that the Rh factor was a fairly good isoantigen for persons lacking it, so that as a result of transfusions, especially repeated trans-

73. Landsteiner and Wiener.[46] Wiener, A. S., and Sonn, E. B.: Genetics **28**:157, 1943.

fusions,[74] anti-Rh agglutinin could be built up in the serum of the person whose erythrocytes were lacking Rh. This at once gave the new factor an importance in transfusion technic which did not seem to be possessed by the M,N series.

The possibilities of isoimmunization, according to the first simple scheme of inheritance proposed, are shown in table 11.

The mechanism[51] by which the damage to the fetus in cases of erythroblastosis fetalis was caused seemed to be this: The Rh antigen,

TABLE 11.—*Possibilities of Isoimmunization in the rh, Rh Series*

	Donor Blood and Agglutinin Possibly Produced by It (Simplified)	
Recipient	rh	Rh
rh	—	Anti-Rh
Rh	—	—

present in the erythrocytes of the fetus (inherited from the father) but absent from those of the mother, passed through the placenta, entering the mother's circulation, and stimulated the production of anti-Rh agglutinins. These agglutinins, diffusing back through the placenta into the fetal circulation, produced the damage characteristic of the disease.

I have already mentioned that it was fairly soon found that the Rh factor was genetically and serologically complex.

According to Wiener's new nomenclature,[67] the principal series of allelic genes concerned in the Rh factor is rh, Rh_o, Rh′, Rh″, Rh_1 and Rh_2. Of these, rh is the Rh-negative gene, and it may be considered, unless an anti-Hr serum (a serum agglutinating red cells that do not contain Rh and certain other types of red cells; see later paragraph) is available, to act as if it were recessive to all the others. Rh_o symbolizes the gene responsible for the antigen detected by the original rabbit and guinea pig anti-Rh serums. It should be noted, however, that this is not the only gene producing an antigen which has the property of reacting with such serums; the property is also possessed by the "double acting" genes Rh_1 and Rh_2 which are alternatively written also with the subscript, o, i. e. Rh_o' and Rh_o''. The genes Rh′ and Rh″ each produce only one of the antigens peculiar to Rh_1 and Rh_2, without any Rh_o. The genes Rh_1 and Rh_2 are thus seen to produce an antigenically complex result, for they cause cells of the persons inheriting them to be agglutinated by anti-Rh′ and anti-Rh″ agglutinins as well as by anti-Rh_o agglutinin. In this respect one may see a similarity to the A_1 gene in the O,A,B series, which produces a type of cell which is agglutinated by anti-A or by anti-A_1. In the O,A,B gene series, however, A_1, if present, represses or conceals the effect of A_2 (i. e., it is dominant), whereas in the Rh series the analogous effect is not observed.

Table 12 shows the frequency of the six Rh genes now known to be present in the human population, as demonstrated in a series of white persons tested by Wiener.

There are twenty-one possible genotypes which may result. Not all of these can be distinguished serologically. The twenty-one genotypes and the phenotypes which present serums (omitting anti-Hr serum) enable one to distinguish are shown in table 13.

It is obvious that the possibilities of isoimmunization here are fully as numerous as those in the O,A,B system. To show all the possible combinations of donor and recipient would require a table which would have $8 \times 8 = 64$ entries. Since such a table would be rather cumbersome, and since the kinds of anti-Rh serums actually met with seem to fall into several well marked classes, I shall merely consider the six kinds of serum

TABLE 12.—*Distribution of Rh Genes in New York's White Population (Based on One Thousand Cases of Wiener)*

Gene	Per Cent
rh	35.9
Rh_o	2.5
Rh_1	43.4
Rh′	1.2
Rh_2	15.7
Rh″	0.4
	100.1

with which workers in this field are at present most familiar. They are shown in table 14.

ANTI-Hr SERUM

There is perhaps still some uncertainty as to the exact nature of the anti-Hr (Rh reversed) serum. First reported by Levine,[75] who suggested that it was directed against the Rh-negative type (rh gene), it was later[76] found to give

74. (*a*) Wiener and Peters.[34] (*b*) Wiener.[45] (*c*) Levine, P.; Katzin, E. M.; Vogel, P., and Burnham, L., in Mudd, S., and Thalheimer, W.: Blood Substitutes and Blood Transfusion, Springfield, Ill., Charles C Thomas, Publisher, 1942, chap. 31.

75. (*a*) Levine, P., and Polayes, S. H.: Ann. Int. Med. **14**:1903, 1941. (*b*) Levine.[35]

76. (*a*) Race and Taylor.[65d] (*b*) Race and others.[65e] (*c*) Race, R. R.; Taylor, G. L.; Cappell, D. F., and McFarlane, M. N.: Nature, London **153**:52, 1944. (*d*) Wiener, A. S.: Personal communication to the author.

positive reactions with several of the antigens produced by Rh genes as well as with that of the rh gene (table 13). The earliest anti-Hr serums apparently were weak or else differed among themselves, for different accounts [77] gave the percentage of positively reacting bloods as any-

SUBTYPES OF RH

The three main agglutinins (not always present without other accompanying agglutinins) available for Rh testing are anti-Rh_0, anti-Rh′ and anti-Rh″. By using them, eight main sub-

TABLE 13.—*Various Genotypes and Phenotypes in the Rh System*

Genotype	Phenotype	Reaction with Standard Agglutinins (85% +) Anti-Rh_0	(70% +) Anti-Rh′	(30% +) Anti-Rh″	Percentage Incidence in New York †	Reaction with Anti-Hr Serum
rh rh	Rh—	—	—	—	12.4	+
rh Rh′ Rh′ Rh′	Rh′	—	+	—	0.8	+ or —
rh Rh″ Rh″Rh″	Rh″	—	—	+	0.3+	+
Rh′ Rh″	Rh′ Rh″	—	+	+	Rare	+
rh Rh_0 Rh_0 Rh_0	Rh_0	+	+	—	2.5+	? +
rh Rh_1 Rh_0Rh' Rh_0Rh_1 Rh′Rh_1 Rh_1Rh_1	Rh_1	+	+	—	53.6	+ or —
rh Rh_2 Rh_0Rh'' Rh_0Rh_2 Rh″Rh_2 Rh_2Rh_2	Rh_2	+	—	+	13.4	+
Rh′ Rh_2 Rh″Rh_1 Rh_1Rh_2	Rh_1Rh_2	+	+	+	16.9	+

† The percentages are based on the results of tests of the blood of 871 white persons tested by Wiener.

where from 30 to 50 per cent. The percentage of negative reactions with a good "standard" anti-Hr serum is about 17.

It now seems that such anti-Hr serums fail to agglutinate, of all the Rh subtypes discussed here, only the red cells of genotypes Rh_1Rh_1, Rh′Rh′ and Rh_1Rh'. The last two are very rare, and Rh′Rh′ is distinguishable by the fact that the red cells do not react with an anti-Rh_0 serum. Since persons of subtype Rh_1 ($rhRh_1$, Rh_0Rh', Rh_0Rh_1, Rh′Rh_1, Rh_1Rh_1) amount to over half the white population (table 13) the anti-Hr serum enables one to differentiate serologically between homozygote and heterozygote in a large section of the population except that one cannot distinguish the rare Rh_1Rh' from the common $rhRh_1$.[78] Without ignoring the rare genotypes, one can identify positively the genotypes of 15 per cent of the population by direct serologic test.[79]

types of Rh can be differentiated; this is shown in table 13.

It should be mentioned that the English workers,[76c] experimenting independently with anti-Rh serums obtained in England, have confirmed the scheme of classification worked out by Wie-

TABLE 14.—*Gene Series rh, Rh_0, Rh′, Rh″, Rh_1, Rh_2: Kinds of (Pathologic) Human Anti-Rh Serum*

No.	Designation	Reacts with Red Cell Antigen Produced by Stated Genes	Percentage of Positive Reactions
1	Anti-Rh_0	Rh_0, Rh_1, Rh_2	85
2	Anti-Rh′	Rh′, Rh_1	70
3	Anti-Rh″	Rh″, Rh_2	30
4*	Anti-Rh_1 (anti-Rh_0″)	Rh_0, Rh′, Rh_1, Rh_2	87
5†	Anti-Rh_2 (anti-Rh_0′)	Rh_0, Rh″, Rh_1, Rh_2	85.5
6	Anti-Hr	rh, Rh_2, Rh″, Rh_0 §	80

* Serum 4 is equivalent to a mixture of 1 and 2.
† Serum 5 is equivalent to a mixture of 1 and 3.
§ Race and others.[76c] Wiener.[76d]

ner, which has just been outlined. Stratton [80] has even detected the very rare Rh′Rh″ subtype, which Wiener had not found among the persons tested by him. As Race and associates pointed out, their arriving at the same scheme suggests the essential correctness of Wiener's scheme, as it would be a remarkable coincidence if two different groups of workers should happen to hit inde-

77. (*a*) Levine and Polayes.[75a] (*b*) Levine and others.[74c] (*c*) McCall, A. J.; Race, R. R., and Taylor, G. L.: Lancet **1**:214, 1944.

78. Race and Taylor.[65d] Race and others.[76c]

79. Wiener, A. S.; Davidsohn, I., and Potter, E. L.: J. Exper. Med. **81**:63, 1945.

80. Stratton, F.: Nature, London **153**:773, 1944.

pendently on the same scheme, complex as it is, unless it were correct at least in its main outlines. Race and Taylor [81] have partially defined still another Rh gene, determined presumably by a special allelic gene which they now designate Rh_z, and which, according to Wiener, is the same as the gene designated by him as Rh_1''. Wiener expressed the belief that such "intermediate" genes exist and that types determined by them have up to now been classified with the usual subtypes. Race and Taylor claim that Rh_z is not an intermediate, as the reactions are all good.

TABLE 15.—*Tests with Anti-Rh_0 Serum* **

Race	Number Tested	Percentage Showing Rh_0 Factor	Percentage Failing to Show Rh_0 Factor
White [82]	334	85	15
American Negroes [82b]	113	99	8
American Indians [82b]	120	99.2	0.8
American Negroes [82a]	264	95.5	4.5
Chinese [82a]	150	99.3	0.7

** The designation is changed to fit the new nomenclature.*

RH AND RACE

The incidence of the Rh factor has been shown to vary in different races.[82] An illustration of this is given in table 15, taken from Levine.[83] It will be seen that the frequency of persons lacking Rh is very low among American Indians and the Chinese. Levine and Wong [82c] stated that, as would be expected, hemolytic disease of the newborn is exceedingly rare among the Chinese.

TABLE 16.—*Tests with Anti-Rh' Serum* **

Race	Number Tested	Percentage Showing Rh_1	Percentage Failing to Show Rh_1
White [81]	334	73	27
American Indians [82b]	60	58	42
American Negroes [82b]	118	46	54
Chinese [82a]	150	93	7

** The designation is changed to fit the new nomenclature.*

When subtypes of Rh are studied, further racial differences emerge, as may be seen in tables 16 and 17.

The distribution of the subtypes of Rh in the white and Negro populations of New York as found by Wiener [82d] is shown in table 17.

Eventually it should be possible to plot the frequencies of the six or seven Rh genes known at present on a map of the world in such a way as to provide perhaps the most valuable contribution to anthropologic classification yet discovered. It has already been found [84] that there are practically no aborigines of Australia and Fiji and no Indonesians [85] whose red blood cells fail to react with anti-Rh_1 or anti-Rh_0 serums.

ACTION OF NATURAL SELECTION

It has been suggested [53] that the white population of Europe (and America) is the result of crossing, within fairly recent times, of at least two stocks, one high and the other low in Rh. It seems possible that natural selection in a population such as ours is constantly tending to reduce the frequency of the Rh-negative gene.

TABLE 17.—*Distribution of Subtypes of Rh in the White and in the Negro Population of New York* **

Series	Number Tested	Percentage Without Rh Factor of Any Type	Percentage Showing Stated Subtype of Rh					
			Rh_1	Rh_2	Rh_1Rh_2	Rh_0	Rh'	Rh''
White	1,000	12.9	54.1	12.8	16.4	2.6	0.9	0.3
Negroes	223	8.1	20.2	22.4	5.4	41.2	2.7	0

** Wiener, Belkin and Sonn.[82d] Wiener, Davidsohn and Potter.[79]*

The way in which "serologic incompatibility" in pregnancy might operate to increase or decrease the frequency of a gene was worked out in Koller's paper, already referred to.[18] See also Wiener,[53] Hogben,[86] Haldane [60] and Fisher, Race and Taylor.[87] Not all workers agree that selection is changing the gene frequencies in the Rh series, however.

FORENSIC APPLICATIONS

If present ideas on the heredity of the Rh types are confirmed, it will be possible to make forensic application of them, and Wiener [76d] has already calculated the probabilities of excluding paternity for the main types, disregarding the rarer possibilities. This is shown in table 18.

The chances of excluding parentage by the use of A, B, O and M and N are about 32 per

81. Race, R. R., and Taylor, G. L.: Nature, London **153**:560, 1944. Race and others.[76c]

82. (*a*) Wiener, A. S.; Sonn, E. B., and Belkin, R. B.: Proc. Soc. Exper. Biol. & Med. **54**:238, 1943. (*b*) Landsteiner, K.; Wiener, A. S., and Matson, G. A.: J. Exper. Med. **76**:73, 1942. (*c*) Levine, P., and Wong, H.: Am. J. Obst. & Gynec. **45**:832, 1943. (*d*) Wiener, A. S.; Belkin, R. B., and Sonn, E. B.: Am. J. Phys. Anthropol. **2**:187, 1944.

83. (*a*) Levine, P.: Science **96**:452, 1942. (*b*) Wiener, Sonn and Belkin.[82a]

84. Simmons, R. T.; Graydon, J. J., and Hamilton, P.: M. J. Australia **1**:553, 1944.

85. Simmons, R. T.; Graydon, J. J., and Ouwehand, C.: M. J. Australia **1**:108, 1945.

86. Hogben, L.: Nature, London **152**:721, 1943.

87. Fisher, R. A.; Race, R. R., and Taylor, G. L.: Nature, London **153**:106, 1944.

cent. The Rh tests raise the chances to about 42 per cent. If one includes exclusions by the subgroups of A and the intraclass Rh exclusions, the chances are raised to about 45 per cent.

IDEAS OF THE ENGLISH SCHOOL

In the opinion of Race,[88] far too much has been written on Rh, and there has been far too much reiteration. It is a great pity that new facts do not turn red in the press. Race and other English workers have written less than the Americans, but some of what they have written shows much more daring, for they have ventured to predict in advance phenomena which ought to be observed in this field.

TABLE 18.—*Probabilities of Excluding Paternity by Rh Tests*

Rh Classes of Father	Probability of Exclusion, per Cent
Rh_0 and Rh negative	29.3
Rh_1 and Rh'	11.3
Rh_2 and Rh''	13.4
Rh_1Rh_2 and Rh'Rh''	14.9
Unknown	15

About two years ago Race and co-workers [76c] had reached independently a scheme of Rh genes which agreed with Wiener's if they brought the notations into line, and this they proceeded to do. In addition to the Rh reactions, Race and coworkers had the advantage of having a strong (standard) anti-Hr serum, which they at first called St. They drew up a table showing how their genes and antiserums corresponded to those of Wiener. In January 1944 R. A. Fisher examined this table and noticed that when a gene was positive with the St serum it was negative with anti-Rh'. He therefore supposed that the antigens disclosed by these two serums were due to alleles, which he called c and C. To the antibodies he gave the corresponding Greek letters, St. becoming γ and anti-Rh' becoming Γ. Fisher suggested that the antigen recognized by the anti-Rh_0, which he called D, probably had an allele d for which the antiserum δ was still to be found. The antigen recognized by anti-Rh'' he called E and he called this antiserum H. He further supposed that there was a hypothetic allele e for which an antiserum η was yet to be found.

In other words, if this theory was correct, there were three tightly linked loci on the chromosome responsible for Rh, making eight possible combinations in one chromosome, as shown in table 19. This scheme was published by Race in a letter to *Nature*, which came out in June 1944.[89] The gene, at first designated as Rh_y, later on proved to have the reactions proper for Rh_z, and the designation was changed accordingly.

Race believes that the two antiserums marked in table 19 as "to be found" are now known, for Mourant [90] has found the one designated as η, and Race believes that the anti-Hr serum described by Waller and Levine [91] has the reactions described by Fisher for δ. Wiener [92] is still inclined to doubt this.

TABLE 19.—*R. A. Fisher's Scheme of Three Linked Gene Loci for Rh, with Genes and Antiserums*

Antigen Present	Genes Present	Antibodies and Their Reactions *					
		Anti-Rh'	Anti-Hr	Anti-Rh_0	Anti-Rh''	Then to Be Found	
		Γ	γ	Δ	H	δ	η
Rh_z	CDE	(+)	(—)	(+)	(+)	(—)	—
Rh_1	CDe	+	—	+	—	(—)	(+)
Rh_y	CdE	(+)	—	(—)	+	(+)	(—)
Rh'	Cde	+	—	—	—	(+)	+
Rh_2	cDE	—	+	+	+	(—)	—
Rh_0	cDe	—	+	+	—	(—)	+
Rh''	cdE	—	+	—	+	(+)	—
rh	cde	—	+	—	—	(+)	+

* Reactions not yet determined serologically or still controversial are included in parentheses. This scheme does not allow for Wiener's "intermediate" genes.

88. Race, R. R.:: Proc. New York Acad., to be published.

89. Race, R. R.: Nature, London **153**:171, 1944.

90. Mourant, E.: Nature, London **155**:544, 1945.

91. Waller, R. K., and Levine, P.: Science **100**:453, 1944.

92. Wiener, A. S.: Personal communication to the author.

Case Reports

LUMBAR VERTEBRAL CHORDOMA

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Chordoma is a neoplasm of rare occurrence arising in vestigia of the fetal notochord. It usually takes the form of a slowly invasive, locally cancerous tumor which only rarely gives rise to metastases.[1] Inasmuch as the fetal notochord becomes incorporated into the vertebral column throughout its length, extending superiorly to the spheno-occipital region, the sites of origin of chordoma are limited to the regions of the vertebral column and the sella turcica. Commonly, however, it is found at either extremity of this area of distribution. Horwitz,[2] in 1941, collected 245 cases from the literature, in 88.2 per cent of which it occurred in the cranial and sacrococcygeal regions. In the remaining 11.8 per cent (29 cases) it was vertebral in origin, and in only 12 of the 29 cases was it confined to the lumbar region. Since then, 1 additional case of its occurrence in this region has been described.[1b]

The exact location of these notochordal vestigia within the vertebral column is not certain. Horwitz,[2] in his study of chordal ectopia, stated that while remnants of the notochord persist within the nucleus pulposus of the intervertebral disks, neoplasia is more likely to occur in those within the vertebral bodies.

From the point of view of histology, chordoma reproduces with remarkable faithfulness the stages in the normal ontogeny of the notochord. While the notochord is of epithelial origin, its cellular structure assumes mesenchymal characteristics, having a purely mechanical supporting function. Thus the tumor may be composed of definitely epithelial structures or may closely resemble spindle cell sarcoma, but in the usual case both types of cell growth may be observed within the same neoplasm. There are similar wide differences in apparent rate of growth and degree of anaplasia in cases of chordoma, the forms varying from the well differentiated tumor containing regular cavities lined by cuboidal epithelium, closely resembling the primitive notochordal tube described by Alezais and Peyron,[3] to the wildly growing anaplastic new growth represented in the case reported here.

The present instance is unusual from several aspects. Not only did the tumor originate in the lumbar vertebral region, giving rise to an intra-abdominal mass of considerable size, but it proved to be one of the most poorly differentiated, actively growing forms of chordoma reported, quite unlike the more common slowly growing, well differentiated one. However, the abundant mucinous matrix, as well as the classic spheroidal vacuolated "physalipherous cell," definitely identifies this tumor as belonging to the chordoma group.

REPORT OF A CASE

A 41 year old Italian railroad worker, married, was admitted to the Boston City Hospital on Aug. 17, 1944 for the second time.

Two years previously he had entered the same hospital with the complaint of a chronic cough of five years' duration, associated with progressive weakness. Physical and roentgenographic examinations and bronchoscopy disclosed abnormalities in the chest which were only somewhat suggestive of bronchiectasis, but he was discharged several days later with a tentative diagnosis of this condition.

When he reentered the hospital two years later he was seeking relief from "pains" in the upper part of the abdomen of ten days' duration. The chronic cough of which he previously complained had spontaneously subsided soon after his earlier discharge, and he had felt entirely well up to the onset of these symptoms referable to his abdomen. The abdominal pain showed frequent remissions and exacerbations without obvious relation to the taking of food or the performance of physical activity. It appeared to be worse at night while he was in bed, but he volunteered the information that this apparent increase in severity might be due to an increased awareness of his discomfort while he was lying idly in bed. The pain occasionally radiated to the left lumbar region but never to the shoulder. While nausea and vomiting were absent, there was frequent gaseous eructation. Concomitantly with the onset of the pain, his appetite had become poor, and he complained of excessive weakness and malaise. There was no associated change in bowel habits nor any observed intolerance to a particular type of food.

He was a well developed, well nourished man, alert and cooperative, showing no signs of a chronic wasting disease. The entire examination yielded results within normal limits save for the abdomen. It was symmetric and not spastic or distended. In the right upper

From the Mallory Institute of Pathology, Boston City Hospital.

1. (*a*) Mabrey, R.: Am. J. Cancer **25**:501, 1935. (*b*) Güthert, H., and Henkel, H.: Zentralbl. f. allg. Path. u. path. Anat. **77**:376, 1941.

2. Horwitz, T.: Arch. Path. **31**:354, 1941.

3. Alezais and Peyron: Compt. rend. Acad. d. sc. **174**:419, 1922.

quadrant, just above and lateral to the umbilicus, there was an easily palpable firm mass, apparently 10 by 10 cm. in area anteriorly, which appeared to be directly under the anterior abdominal wall but not attached to it or to the liver. It was not tender, not fluctuant and not expansile; it was slightly nodular. The liver's edge, palpable approximately 1 cm. below the costal margin, was sharp and smooth. There was no tenderness of the costovertebral angle, nor were there any palpable lymph nodes in the inguinal, cervical or clavicular areas. Roentgenograms of the abdomen did not reveal any soft tissue masses. A Graham series, made to visualize the gallbladder, demonstrated a faintly filled but not enlarged bladder, which contracted poorly after a fatty meal.

The hemoglobin content was 60 per cent, with a red blood cell count of 3,280,000 cells per cubic millimeter. The white cell count was within normal limits in number and types. The total protein of the serum was 6.51 mg. per hundred cubic centimeters.

After five days of hospitalization an exploratory laparotomy was performed. The peritoneal cavity contained approximately 5 liters of a serosanguineous fluid, removal of which revealed a slippery mucinous material covering most of the serosal surfaces. The region of the transverse mesocolon was filled with solid tumor tissue, which was fused to the stomach, the pancreas, the transverse colon and the left kidney. This mass extended posteriorly about the root of the mesentery of the small bowel and completely enveloped the aorta and the vena cava in the upper lumbar region. No metastases were found in the liver or the lymph nodes. Two biopsy specimens were taken from the more solid areas of the mass, and the abdomen was closed without definite knowledge as to the primary site of the lesion.

The patient's postoperative condition was poor, with progressive abdominal distention and respiratory embarrassment for the succeeding two days. He died quietly on Aug. 26, 1944, nine days after his entry into the hospital and approximately nineteen days after the onset of symptoms relative to this present illness. The clinical diagnosis at discharge was carcinomatosis, primary site unknown. The biopsy specimens consisted of a clear, white, mucoid tissue, essentially identical grossly and microscopically with the tumor obtained at autopsy.

Postmortem Examination.—Externally the body was that of a well developed and well nourished, moderately obese white man, appearing approximately his stated age of 41 years. There was no demonstrable edema or lymphadenopathy.

The remainder of the external examination, as well as the investigation of the pleural and pericardial cavities, disclosed no pathologic change save congestion and edema of the lungs with scattered 0.5 cm. raised areas of gray consolidation in the lower lobes. The abdomen was slightly distended and showed an 18 cm. incision splitting the right upper rectus muscle, closed by silk and wire sutures. The margins were sealed with a fibrinous exudate which gave no evidence of suppuration. The peritoneal cavity contained 1 liter of a sanguinomucoid fluid, in which floated strings of mucus. The mass previously described in the operative notes was found to be 15 by 18 cm. in area as it presented anteriorly. It was matted together with the omentum and was intimately adherent to the greater curvature of the fundic portion of the stomach as well as to the transverse colon. On the right side it seemed to envelop the ascending colon without actually narrowing the lumen or penetrating its wall. On the left, the tumor was densely adherent to the spleen and the kidney. The adrenal gland was apparently buried within it. On section later, the adrenal gland was clearly definable and grossly was not invaded by the neoplasm. the latter extended posteriorly about the pancreas and the root of the mesentery of the small intestine into the retroperitoneal tissues and appeared to encircle the great vessels completely. It was densely adherent to the prevertebral fascia in the region of the second and third lumbar vertebrae. However, the vertebral bodies were well formed, not collapsed, with no demonstrable bony defects or erosions. The intervertebral disks were within normal limits, with no narrowing of the spaces. Sections through the more solid portions of the tumor mass revealed an almost pure white, glassy surface, covered with a tenacious, glairy mucus which pulled out into long, filamentous strands, so copious in amount that they layered the instruments, hands and dissecting table. Investigation of all the contiguous organs showed that the tumor tissue extended up to the external limits of these structures without gross invasion of any of them. The pancreas, while practically surrounded by the tumor, was nonetheless structurally intact. The mucosa of the entire gastrointestinal tract was uninvolved.

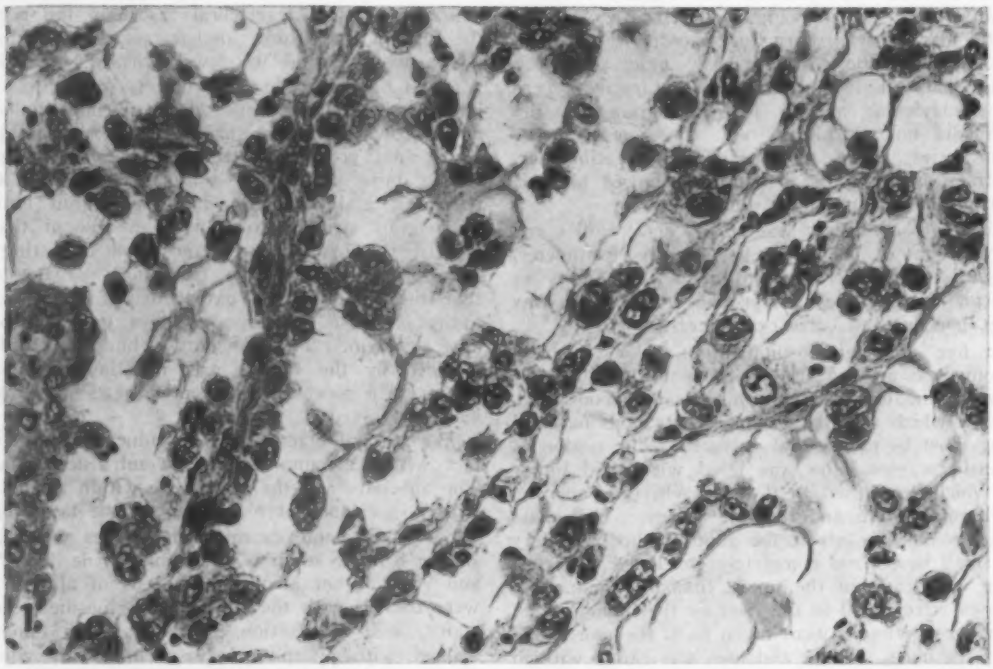
The adrenal glands and the kidneys, though in contact with the tumor, were not intrinsically involved. The appendix and the liver were within normal limits, with no evidence of metastases in the latter organ.

For microscopic examination, tissues were fixed in Zenker's fluid, solution of formaldehyde U.S.P. 1:10 and 95 per cent alcohol. Sections of all the organs were treated with the routine phloxine–methylene blue stains, and, in addition, portions of the tumor were stained with phosphotungstic acid–hematoxylin, Mallory's aniline blue stain, Best's carmine for glycogen, and thionine, as well as with Mayer's mucicarmine and acid-hematein stains for mucin.

The tumor was remarkable histologically in two respects: first, in the degree of anaplasia demonstrated by the tumor cells, and second, in the large amounts of mucinous matrix between the cells, comprising roughly one half to two thirds of the entire bulk of the tissue. The cellularity varied, however, from field to field so that in many areas the cytologic elements were more abundant than the matrix. The tissue, on the whole, was viable, with only scattered microscopic foci of recent hemorrhage and infarct-like necrosis. The tumor cells were seen rather widely separated, lying singly, in syncytium-like masses or even as irregular, loose cords, with the intervening spaces filled by an abundant mucoid matrix containing a loose network of fibroblasts (figs. 1 and 2). In certain areas the cells appeared to be actually floating in a sea of mucin. Their size varied from that of a polymorphonuclear leukocyte to the dimensions of multinucleate giant cells four and five times as large. This variability in size was accompanied by an equally great pleomorphism, so that areas of epithelium-like cells varying in shape from spherical to polygonal to reniform could be seen, interspersed here and there by areas where the cells assumed the spindle shape characteristic of sarcoma. The abundant cytoplasm of the cells, strung out at times into bizarre-shaped processes, was acidophilic and finely granular. In most cells it was homogeneous; however, not uncommonly, large clear single or multiple vacuoles could be found, which in rare instances pushed the nucleus of the cell to the periphery, producing so-called signet ring forms. "Physaliferous cells," while present, were not numerous and were seen as distended spherical or ovoid cells containing multiple cytoplasmic vacuoles. Often this vacuolation created perinuclear

clear spaces, traversed only by fine cytoplasmic strands, and in yet other cells it appeared to displace the cytoplasm completely so that only cell membranes and nuclei were visible (fig. 3).

masses (fig. 4), occasionally presenting apparent pseudopodia. They were characteristically vesicular, with darkly staining nuclear membranes and finely divided chromatin, and contained usually two nucleoli,



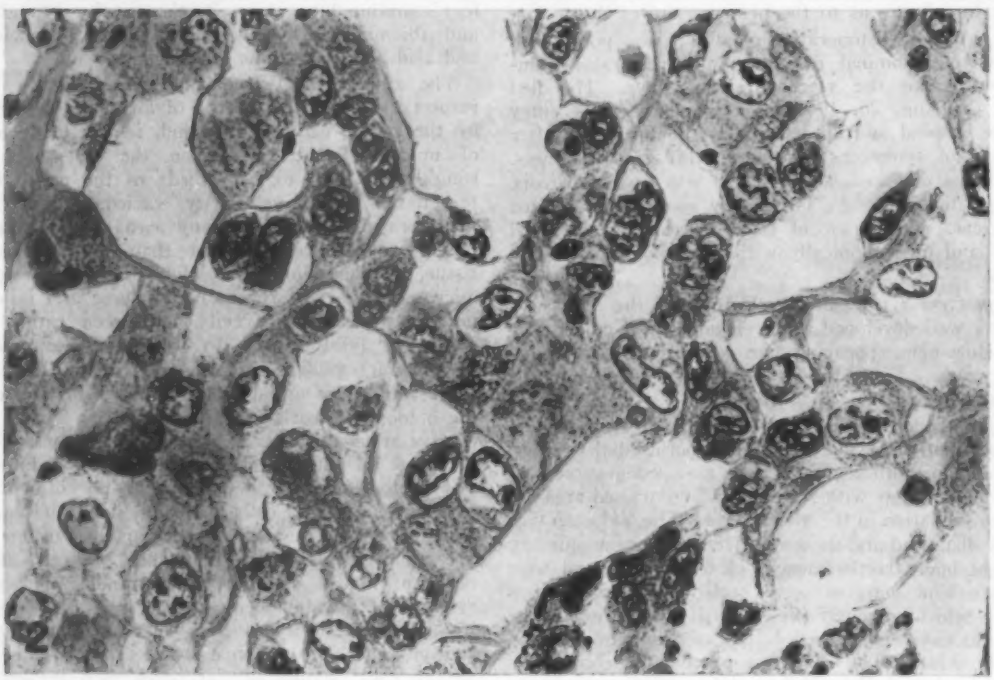


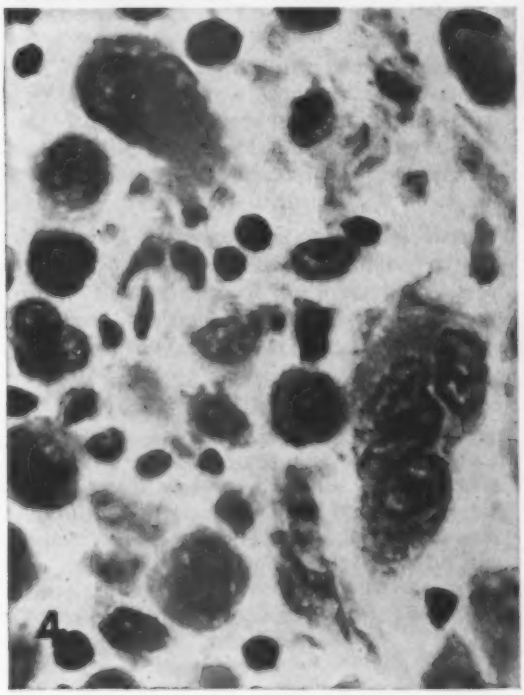
Fig. 1.—A low power view of the tumor showing the cells lying singly, in cords and in masses, separated by wide spaces containing mucin.

Fig. 2.—A more detailed view of the tumor structure showing the cellular pleomorphism and the variation in size.

The nuclei of the cells were equally striking in their pleomorphism, varying from simple small round or reniform shapes to giant polymorphous nuclear

sometimes one. As many as five nuclei could be counted within some of the giant cells. Mitotic figures were frequent and bizarre (fig. 5), especially in

some of the tumor giant cells. Unlike the chordoma described by Peers,[4] this one revealed no intracellular fibrils. However, using Best's carmine stain, numerous cells, particularly the large vacuolated ones, were shown to contain granular glycogen. The extensive masses of mucin between the cells stained readily with thionine and Mayer's acid-hematein, although in scattered areas the mucin apparently took the stain less well than in other areas. No mucin could be demonstrated intracellularly—an observation in agreement with many previous reports.



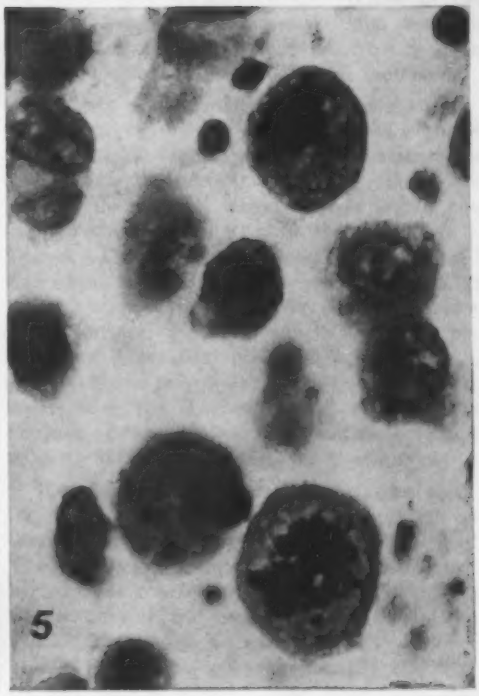


Fig. 3.—A diagnostic vacuolated distended "physalipherous cell."
Fig. 4.—Two bizarre giant cells showing the polymorphous character of the nuclei.
Fig. 5.—Cells in atypical mitotic division.

No tumor metastases were found. Microscopic examination of the organs contiguous to the mass showed

4. Peers, J.: Am. J. Cancer **32**:221, 1938.

that uniformly the tumor extended up to these organs and at times infiltrated between layers and within the supporting stroma without actual destruction of the tissue. Hence it was found in the subserosal fat and between the muscular layers of the stomach and the transverse colon, within the fibrous stroma of the pancreas and in the perirenal and periadrenal fat.

COMMENT AND SUMMARY

Owing to the rarity of chordoma occurring in the lumbar vertebrae, only 13 cases having been described previously, the diagnosis in the present instance was not suspected either clinically or at operation. In fact, it was only when microscopic sections were available after autopsy that the true nature of the neoplasm was appreciated. Perhaps if the diagnosis had been suspected during the postmortem examination a more careful search might have revealed some small defect in the vertebral column as the exact site of origin of this tumor. That the defect may be insignificantly small was amply demonstrated experimentally by Ribbert [5] in 1894, when he produced so-called tumors in rabbits by simply puncturing intervertebral disks with a needle, permitting the escape of notochordal remnants through these tiny apertures, with the resulting development of tumors closely resembling chordoma.

Microscopically, the presence of the only two constant features of chordomas,[6] namely, abundant mucinous matrix and large vacuolated "physalipherous cells," identify this tumor as belonging to this category. It is of interest to note that despite the cellular anaplasia and mitotic activity, indicating apparent rapid growth in this tumor, it was only locally cancerous, with no metastases and only a slight tendency to infiltrate the surrounding structures. This apparent disparity of histologic cancer and clinical invasiveness is a characteristic of the tumors diagnosed as chordoma rendering their pathologic recognition of more than academic importance, since once the diagnosis is established, radiation can be effectively used to control the growth at the primary site, with the reasonable assurance—especially in the lumbar tumors—that metastases will not develop.

5. Ribbert, H.: Verhandl. d. Cong. f. inn. Med. **13**: 455, 1895.

6. Stewart, M. J., and Morin, J. E.: J. Path. & Bact. **29**:41, 1926.

CONGENITAL DIAPHRAGMATIC HERNIA IN THE DOMESTIC CAT

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Diaphragmatic hernia in man has received considerable attention in the literature and is well understood both in its etiologic aspects and in its various morphologic manifestations. Bremer [1] has described the development of the human diaphragm, pointing out the danger of transferring the findings relative to development in other animals to man without observational basis, and relating the common forms of congenital diaphragmatic hernia to the imperfect completion of the various steps of the normal developmental sequence. The number of instances in which congenital hernia of a domestic animal has been reported is small. The paucity of records does not necessarily indicate a lower incidence among domestic animals, for in domestic animals hernia often escapes observation. That congenital hernia will be observed oftener in domestic cats than in many other species is to be expected since large numbers of cats (Felis domestica) are dissected annually in undergraduate courses in biologic science. The vast field of comparative embryology is, as Bremer said, "almost unexplored," and since congenital defects have origin in embryogeny, extensive information concerning the nature of such defects has direct application in a study of the broader question. Further instances of congenital hernia occurring in the domestic cat or in any other domestic or laboratory animal will be of sufficient significance to warrant description.

Reports of 4 instances of congenital hernia of the domestic cat have been encountered in the literature.[2] The purpose of the present paper is to describe a fifth instance and to examine all 5 cases for purposes of comparison.

DESCRIPTION

One opening 20 mm. in diameter was located in the left wing of the diaphragm ½ inch (about 1 cm.) ventrolateral to the esophageal hiatus. The opening perforated the left anterior tendinous projection of the central tendon and encroached on the left sternocostal (muscular) portion along the medial border of the latter. Except for this encroachment the muscle of the left sternocostal portion was complete. The esophagus followed its normal course in the thorax and passed into the abdominal cavity through the esophageal hiatus, at which point it was directly continuous with the stomach as usual. The stomach was reduced to 2½ inches (6.5 cm.) in length from the cardiac to the pyloric sphincter and was totally recurved on itself along its lesser curvature so that it exhibited a U-shaped bend one third of the distance from the cardiac sphincter. The caudal two thirds of the stomach thus passed anteriorly through the hernial hiatus of the diaphragm and into the thoracic cavity. The greater curvature was thus accentuated, and the stomach appeared on superficial examination to be a mere protuberance of some thoracic structure on the left side of the abdominal cavity. The stomach was tightly constricted where it passed through the diaphragmatic schism. The duodenum passed to the right from the pyloric sphincter within the thoracic cavity, then became convoluted and continued into the remainder of the small intestine which, with its mesentery, lay entirely within the thorax. The cecum and 1 inch (2.5 cm.) of the colon also occurred within the thoracic cavity, the ileum entering the cecum from the right. The long axis of the cecum was anteroposteriorly oriented, and from the cecum the colon passed caudad in almost a straight line through the schism and directly to the rectum in the pelvic cavity. The ascending, transverse and descending portions of the colon were thus not grossly defined, but the entire colon retained its dorsal mesentery.

The liver was located almost wholly within the abdominal cavity but lay entirely on the right side, and the normal left-right axis was rotated about the longitudinal axis through 90 degrees so that a line drawn through the original left-right axis would now lie in the dorsoventral plane, with the left lobes ventrally located. This rotation had been brought about by pressure from the colon as it was dragged anteriorly through the hiatus. The liver exhibited a longitudinal depression for the colon on its medial aspect. The left lobes were about one-third natural size. A small portion of the left superior lobe protruded through the aperture in the diaphragm and occurred within the thoracic cavity. The pancreas and the spleen, the latter reduced in size, lay within the thoracic cavity.

The abdominal contents occupied the right half of the thoracic cavity, the mediastinal structures having been pushed cephalad and to the left of the midline. The left lung was much smaller than normal and lay high in the thoracic cavity, while the right lung was normal in size and occurred lower in the cavity than usual. There was an extensive adhesion between the middle lobe of the left lung medially and the heart on its left lateral aspect. The heart was considerably reduced in size. The abdominal parietal peritoneum did not appear to be continuous within the thoracic cavity. The animal had not noticeably suffered from malnutrition as far as postmortem observation could reveal, but it is highly probable that food had to be taken in small amounts and often. The case herein described resembles that described by Plagens and

Contribution 71 from the Department of Zoology, Physiology and Entomology, Louisiana State University.

1. Bremer, J. L.: Arch. Path. **36**:539, 1943.

2. (*a*) Briscoe, G.: J. Anat. **62**:224, 1928. (*b*) Plagens, G. M., and Bradbury, J. T.: Anat. Rec. **46**: 259, 1930. (*c*) Rifenburgh, S. A.; Lawson, W. P., and Ogden, R. P.: Proc. Indiana Acad. Sc. **47**:285, 1937. (*d*) Day, C. D.: J. Tennessee Acad. Sc. **15**:267, 1940

Bradbury [2b] with the exception that in the latter case the mediastinum was pushed to the right rather than to the left.

COMMENT

In 3 of the 4 American cases of congenital hernia of the cat the anomaly occurred in a male while in the case described by Day [2d] it occurred in a female. Maximum displacement of the abdominal contents in congenital hernia of the domestic cat evidently transposes to the thoracic cavity the entire alimentary canal from the cardiac portion of the stomach to the first inch or more of the colon, along with the pancreas and the spleen, but excludes most of the liver. Less extensive prolapse incorporates the stomach, anterior portions of the small intestine, and the pancreas and the spleen in the thorax and excludes the posterior portions of the small intestine and all of the large intestine, in which case the normal configuration of the colon within the abdominal cavity is exhibited. Still less extensive prolapse incorporates the pancreas and the spleen within the thoracic cavity, provided part of the greater omentum has prolapsed, but excludes the stomach and portions of the duodenum, jejunum and ileum. In minimal congenital displacement a tubular hernial sac protrudes into the thoracic cavity and the peritoneum may be everywhere continuous. Inside the prolapsed sac may occur one lobe of the liver and several inches of the greater omentum.

The extent of prolapse is not always proportional to the size of the schism. The diaphragm may be almost absent and yet less thoracic displacement of abdominal viscera may be exhibited than in other instances in which the aperture at time of death is much restricted. In any case the liver seems typically to be excluded from the thoracic cavity.

Present reports seem to indicate involvement primarily of the semilunar tendon as defined by Reighard and Jennings,[3] with the rupture occurring in a central location (Briscoe [2a]) or in the left horn of the central tendon (present case and that of Plagens and Bradbury [2b]), in which case the muscular elements of the left half of the diaphragm may or may not be lacking. The rupture reported by Rifenburgh and coworkers [2c] involved the right side of the diaphragm dorsally. In the case reported by Day [2d] there appeared to be marked developmental failure both of the muscular portion and of the septum transversum. Bremer [1] stated that in man the central tendon does not seem to be affected. The thoracic viscera in the cat may be pushed either to the left or to the right as a result of a herniation through the left wing of the diaphragm.

3. Reighard, J., and Jennings, H. S.: Anatomy of the Cat, ed. 3, New York, Henry Holt & Company, Inc., 1934, p. 151.

PHEOCHROMOCYTOMA AND SUDDEN DEATH FOLLOWING INJURY OF THE HEAD

Report of a Case with Autopsy

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Adrenal tumors are divisible into those which produce endocrine substances and those which do not. The only cells of the adrenal medulla known to produce a hormone are the chromaffin cells, or pheochromocytes, and the only endocrine substance which they are known to produce is epinephrine.[1] Tumors of these cells are known as pheochromocytoma, are usually noncancerous and are considered rare. Because of their ability to produce epinephrine, sometimes in large quantities, with clinical systemic effects, these tumors have been termed "functional" by Karsner [2] and have been the subject of fairly detailed study, both clinically and at necropsy, in those instances in which they have been encountered. Although rare, the clinical picture of paroxysmal hypertension, paroxysmal tachycardia, vasomotor disturbances and refractory glycosuria, which constitute the most prominent features of the syndrome, have been studied and reported fully.[3] In the limited available literature, however, few details are given concerning sudden death associated with the presence of pheochromocytoma. The following report of a case is presented, not as a contribution to the clinical study of tumors of this type, but rather as an interesting forensic consideration of a case of sudden death.

REPORT OF A CASE

A 24 year old white man was admitted to a Station Hospital on Dec. 5, 1942 at 9:30 in the evening, because of a laceration of the forehead. He had been struck with a club. Although momentarily stunned, he had not lost consciousness and had remained on his feet after the blow. Examination on admission revealed a laceration 2 cm. in length over the midline of the forehead, which was cleansed and sutured. The rest of the examination produced nothing remarkable; no abnormal reflexes were found, and the blood pressure was 118 systolic and 70 diastolic. There was an odor of alcohol to the patient's breath, but he showed no clinical signs of intoxication. He was seen on rounds daily, and on the second and third hospital days a neurologic examination gave negative results. The blood pressure was 118 systolic and 70 diastolic on the second day and 124 systolic and 70 diastolic on the third. The record of progress subsequently indicated that the patient was asymptomatic and up and about. On the fifth hospital day he was seen during rounds at about 8 a. m., at which time he offered no complaints. Following this, he repaired to the bathroom "to bathe and shave." It is not known whether he was at stool while in the bathroom, but at about 8:15 he complained to another patient in the bathroom that he felt faint and weak and had "gas on the stomach," broke out in a "cold" perspiration and went to bed. The nurse was notified, and on reaching him, found him dyspneic and cyanotic, complaining of pain in his chest and coughing up a bloody froth. The ward physician was called, but despite the administration of oxygen, morphine, epinephrine and finally artificial respiration, the patient died at 9:20, about one hour after the onset of the attack. The blood pressure was not recorded during the terminal episode.

On admission the routine laboratory work had given the following data: The urine was amber in color and acid; the specific gravity, 1.028. It contained no sugar or albumin, and microscopic examinations gave negative results. The red blood cell count was 4,900,000, the hemoglobin value 85 per cent, and the white cell count 11,800, with stab forms 2, polymorphonuclear leukocytes 76 and lymphocytes 22 per cent. The Kahn test was negative.

Autopsy (approximately five hours after death).—The body was that of a 24 year old white man, 66 inches (167.5 cm.) tall and weighing about 165 pounds (74.5 Kg.). The nutrition and the muscular development were good. There was moderate rigor mortis, and dependent lividity was present over the back. The head, the neck and the nail beds were intensely cyanotic, and a fine white foam was present on the lips.

There was a healed laceration of the frontal region of the scalp at the hair line, about which the hair had been shaved. It was 2 cm. in length and vertical in position, and contained three silk sutures in situ, with no evidence of infection. There was no other evidence of trauma about the head.

The scleras and conjunctivas were clear; the pupils, round and equal, in middilatation. There was no discharge from the external auditory canals and no ecchymoses about the ears or the regions of the mastoid processes. There were no abnormalities of the nose. There was a fine white froth on the lips. There was no blood in the mouth, and no abnormalities of the oral cavity. There was no adenopathy.

The chest was of normal configuration. Before the pleural cavities were opened, the diaphragm was located at the level of the fifth rib on the right and at the level of the fifth intercostal space on the left. When the chest was opened, the organs were found in normal relations, and the pleural cavities were free of adhesions and fluid.

Both lungs were normal in position, deep purplish blue and voluminous, filling both pleural cavities. The pleural surfaces were smooth, and there was absence of normal crepitation except at the very edge of the

1. Cahill, G. F.: Pennsylvania M. J. **47**:664, 1944.
2. Karsner, H. T.: Human Pathology, Philadelphia, J. B. Lippincott Company, 1942.
3. (*a*) Belt, A. E., and Powell, T. O.: Surg., Gynec. & Obst. **59**:9, 1934. (*b*) Bodansky, M., and Bodansky, O.: Biochemistry of Disease, New York, The Macmillan Company, 1940, p. 302. (*c*) Engel, F. L.; Mencher, W. H., and Engel, G. C.: Am. J. M. Sc. **204**:649, 1942.

basal margins. Dissection of the bronchial tree revealed it to be filled with a fine white foam, but no foreign body or obstructive lesions were found. The pulmonary vessels revealed no thrombi or emboli. On section, the lungs were found to be diffusely cyanotic, and the surfaces were wet and dripped a clear fluid even without pressure other than that necessarily used. No signs of localized consolidation were found.

The heart was normal in size, shape and position. The pericardium was smooth and glistening, and there was no free fluid in the pericardial sac. The epicardial fat was moderate in amount. The myocardium was purplish brown, was not grossly hypertrophied and on section revealed no abnormalities beyond the cyanotic hue. The ventricular cavities were normal in size and contained no thrombi, the valves were thin and delicate, and the chordae tendineae were not thickened or fused. In the middle of the anterior mitral leaflet was a minute atheromatous plaque about 1 mm. in diameter. The ring of attachment of the valve was not thickened. The endocardium was normal. The coronary vessels were patent throughout, but the right coronary artery presented a minimal degree of intramural atherosclerosis involving about two thirds of its circumference for a distance of about 4 mm., beginning at a point 1 cm. from the origin of the vessel. There was no encroachment on the lumen of the coronary vessel. The coronary ostiums were patent, although the aorta presented a mild degree of atherosclerosis in the region of the ostiums. The aorta presented a minimal degree of diffusely scattered atheromatous plaques throughout its ascending and descending portions, both thoracic and abdominal.

The thyroid gland was normal. The thymus could not be found in the anterior mediastinal tissues.

The abdomen presented no superficial abnormalities and on section revealed a normal amount of subcutaneous fat. The peritoneum and the peritoneal cavity were normal and contained no fluid, and the abdominal organs presented normal relationships. The esophagus was normal, the stomach presented mild congestion, with injection of the submucosal blood vessels, and postmortem autolysis had started. The duodenum was normal; it contained a semifluid green chyme. The papilla of Vater was normal. Sections of the rest of the intestinal tract were normal. The appendix was present and not remarkable. The pancreas was normal.

The liver was dark purplish brown and did not descend below the costal margin. On section the structure appeared normal; the venous tree was markedly engorged. The capsule was normal.

The gallbladder was normal in size, shape and position. The ducts were patent. The bladder emptied readily on pressure. Section revealed no abnormalities.

The spleen was normal in size, shape and position. The capsule was normal. On section the pulp was of a cyanotic hue and appeared moderately congested.

The kidneys were normal in size, shape and position. The capsules were normal and stripped with ease, revealing smooth surfaces. On section the renal structure was well preserved and normal in appearance except for a cyanotic hue. The pelves, the ureters and the renal vessels were normal. The bladder contained 300 cc. of yellow urine and presented no abnormalities. The prostate was not remarkable. The testicles were normal.

The left adrenal gland was normal in appearance. The right adrenal gland was represented by a small cap of what appeared to be a fragment of normal adrenal tissue surmounting a round, soft tumor, measuring about 3.5 cm. in diameter. This tumor was almost cystic in its softness, was well encapsulated and on section presented a deep pink homogeneous surface of semisolid consistency and no definable structure.

The extremities showed deeply cyanotic nail beds; there were no deformities, no clubbing of the digits and no edema.

The calvarium was normal and presented no fractures. On removal of the brain two fissure fractures were seen in the posterior third of the left middle fossa, over the petrous pyramid. These fracture lines were each 1 cm. in length, parallel to each other, 1 cm. apart, and parallel to the posterior margin of the middle fossa. There was no depression of bone, and examination of the underlying region of the mastoid process gave negative results. The fractures were so fine as to be almost indistinguishable from the adjacent suture lines.

The dura mater was normal in appearance and was removed without difficulty. No subdural hemorrhages were found. The dural sinuses were free of thrombi. The brain revealed no evidence of gross edema, but over the inferior aspect of the right frontal pole was a small ecchymosis measuring 0.5 by 0.5 cm. Over the inferior aspect of the left temporal lobe, in the region of the fractures in the left middle fossa, was a slight fibrinous roughening of the pia-arachnoid, but no hemorrhages or damage to the underlying brain could be discerned. Serial sections of the brain, the brain stem and the upper part of the spinal cord revealed no hemorrhages or other abnormalities. The blood vessels at the base of the brain were normal, and there was no increase in the amount of cerebrospinal fluid within the ventricles or in the subdural spaces. The hypophysis was grossly normal.

Unfortunately, the autopsy was not done under optimal circumstances and the weights of the organs were not obtainable.

Microscopic Examination (First Lieutenant C. F. Vorder Bruegge, Medical Corps, Army of the United States).—(*a*) Heart: The epicardium and the endocardium were smooth and regular. The muscle bundles showed some separation by edema fluid, and there were several small foci of infiltrating small round cells. These cells lay scattered among the muscle fibers either singly or in small nests. There was some fragmentation. Many small arteries showed slight thickening and irregularity of the intima. A section including the base of the aorta exhibited some irregularity of the intima, and at one place there was considerable thickening. This place had a somewhat foamy appearance from vacuolated cells and areas of lightly stained homogeneous pink material with a lacy appearance. A few small round cells had infiltrated this region and one nest of similar cells with a few eosinophils was noted in the deeper layer of the muscularis.

(*b*) Lungs: The pleura was for the most part even and thin, though there were several areas of moderate thickening. A few scattered deposits of black coal pigment were noted. In almost all areas studied the alveoli showed marked congestion of the capillaries, and the alveoli were completely filled with a homogeneous, noncellular pink-staining material, in which were enmeshed numerous large pigment-laden macrophages, occasional polymorphonuclear leukocytes and a few mononuclear cells. Near the periphery were scattered areas of partially opened alveoli, but even here there was some collection of edema fluid with infiltrations of white blood cells and large macrophages. The bronchi were filled with a similar material, and the submucosal layer of many bronchi exhibited nests of

lymphocytes. There was some edema of the bronchial mucosa. Many arteries seen in these sections showed thickening of the intimal layer.

(*c*) Liver: The capsule was thin and smooth. The central veins were somewhat dilated, and the sinusoids in these regions were also more widely patent than usual. The liver cells had a foamy appearance from numerous small vacuoles, and there were scattered large vacuoles which pushed the nuclei peripherally in the cells. Cells of liver cords near the central veins frequently contained deposits of light golden brown pigment, and there were scattered deposits of bile pigment. The bile ducts were of normal diameter, and their structure was not unusual. About some biliary radicles there were a few infiltrating small round cells and eosinophils. Scattered small blood vessels were markedly dilated.

(*d*) Spleen: The capsule was of normal thickness and was even and regular. The malpighian bodies varied in size and outline. The sinusoids were dilated, and there was considerable congestion throughout. The central arterioles were for the most part markedly narrowed by intimal thickening and hyalinization. Throughout the pulp many phagocytic cells had taken up deposits of light brown pigment.

(*e*) Pancreas: There were moderate congestion, edema and autolysis. The islets of Langerhans were easily identified and had fairly sharp demarcation. There were scattered areas in which cellular detail was indistinct, but these changes were thought to be principally those of autolysis and of preparation of the tissues. The pancreatic ducts were not remarkable. The acinous cells were intact and deeply stained.

(*f*) Gastrointestinal Tract: The mucosa of the stomach was intact, and in this area a large number of parietal cells was exhibited. There were considerable congestion and edema both in the lamina propria and in the deeper layers of the wall. Small aggregates of scattered lymphocytes were noted in the deeper layers of the mucosa. The muscularis was not remarkable. The small intestine exhibited similar changes of congestion, edema and autolysis.

(*g*) Kidney: The fibrous capsule was thin and even. The glomeruli showed marked congestion of the capillaries in the glomerular tufts, and throughout the kidney congestion of the vascular system was marked; this was especially striking in the capillaries lying among the collecting tubules. In some glomerular capsules there were small amounts of granular, noncellular pink-staining material. Autolytic changes were noted in the lining epithelial cells of the convoluted tubules, and these cells were not sharply demarcated. These tubules contained bits of cellular debris and wisps of noncellular pink-staining materials. Several sections included the epithelium of the renal pelvis and areas of pelvic fatty tissue. No structural abnormalities were noted.

(*h*) Prostate: There were the usual fibromuscular stroma and closely placed acini of irregular shape with numerous finger-like infoldings into the lumens. This epithelium was tall, columnar and everywhere irregular, although its thickness varied slightly. Scattered acini contained rounded, dense, dark, deep-staining concretions. There was considerable congestion of some areas, and several acini were surrounded by an irregular zone of infiltrating lymphocytes. A few scattered neutrophils were present.

(*i*) Testes: The testes were normal.

(*j*) Bladder: There were congestion and edema of the muscularis.

(*k*) Skeletal Muscle: This was normal.

(*l*) Hilar Lymph Node: The capsule of the node was thin and intact. The peripheral lymphatic sinus was patent in many areas, but in some its lumen could not be made out. There were scattered lymphoid follicles, some showing small, indefinitely outlined, active germinal centers. This node exhibited marked congestion and considerable edema, which somewhat altered the usual appearance. Rather numerous eosinophils and plasma cells were noted in the pulp.

(*m*) Brain: The meningeal coverings were thin and intact in these sections. There was moderate congestion of the superficial blood vessels, and throughout the brain sections there were dilatation and congestion of small capillaries. The brain tissue was generally intact but showed changes of autolysis. The Virchow-Robin spaces were wider than usual, but there was no necrosis or extensive destruction of brain tissue. In the region of the right frontal lobe there were several minute areas where small numbers of red cells lay apparently free in the brain tissue.

(*n*) Pituitary Gland: The usual lobes of this gland were easily identified, and the normal cell components were present. Congestion, autolysis and edema were noted. There appeared to be an increased number of acidophilic cells in the anterior lobe. In the pars media, there were numerous small cystic spaces containing a homogeneous pink-staining material, and there was one rather large cystic space filled with the same material. These spaces were surrounded by a columnar layer of cells, though the larger spaces had a flattened cellular lining.

(*o*) Adrenal Glands: The left adrenal gland had a normal outline, and the usual layers were well demarcated. Congestion was noted throughout the gland, and there were slight autolysis and edema. The capsule was thin and regular, and the cortex exhibited the usual type of cells. The medulla exhibited the changes already mentioned, and no neoplastic tissue was observed. The right adrenal gland was altered by the neoplastic growth described grossly. The relatively normal gland was found fitted like a cap over the neoplastic tissue. Over the superior portion of this gland there was a thin fibrous capsular layer. The usual cortical cells were identified and did not appear abnormal except that immediately beneath the capsule the zona glomerulosa exhibited occasional small cystlike spaces. The gland was somewhat flattened, and the usual medullary layer was not found, though some medullary tissue was present. In the center of the gland there were numerous markedly dilated blood vessels, and several sections showed large thrombi. Several of these thrombi had been almost completely organized by fibroblasts, which were noted growing into the homogeneous pink-staining material of the thrombus. Scattered red and white blood cells were noted. Lying underneath the adrenal tissue described was the tumor mass referred to grossly. It was completely surrounded by a thick connective tissue capsular layer and presented a similar appearance in all sections. There was a diffuse growth of fairly large cells having large oval nuclei. The cells varied in outline, were somewhat loosely arranged and appeared to have been somewhat shrunken in the staining process. For the most part, these cells were polygonal, but the outline varied, and in some regions the cells became rather spindle shaped or elongated. The cytoplasm appeared faintly eosinophilic and slightly opaque. Minute vacuolated spaces were noted in the cytoplasm of some cells, and in a few places small

amounts of light brown pigment were noted. Homogeneous acidophilic cell inclusions were noted. At several points throughout the tumor there were giant cells, which occasionally had multiple nuclei and more darkly staining cytoplasm than the surrounding cells. There were scattered dilated capillaries.

Final Diagnosis.—Pheochromocytoma of the right adrenal gland and thrombi of the veins of the medulla of this gland, with partial organization; marked bilateral pulmonary edema and congestion; healed frontal laceration of the scalp; fissure fractures, of the left middle fossa of the skull; general congestion and autolysis of the brain; minimal petechial hemorrhages and slight edema of the right frontal lobe; small cyst in the pars intermedia of the pituitary gland; cardiac edema and congestion; slight atherosclerosis; congestion and edema of viscera.

COMMENT

The sudden and dramatic death following four days lacking in premonitory signs or symptoms poses three questions: Was the injury of the brain the cause of death? Was the pheochromocytoma the cause of death? Was there a relation between the injury of the brain and the adrenal tumor? These will be discussed in order.

Although a person dying from craniocerebral injury might reveal little at autopsy, this is not the usual finding, and the presumption that death was due to the injury would be warranted only in the absence of any other plausible reason for death. Ordinarily with death from craniocerebral injury, i. e., trauma of the brain, there is revealed a demonstrable amount of gross injury, such as cerebral edema, with flattening of the convolutions, and hemorrhage [4] or more obvious pathologic conditions, such as subdural or epidural hematomas. In this case the findings in the brain were suspiciously minimal, being limited to the small superficial ecchymosis of the right frontal pole and the two fissure fractures in the left middle fossa of the skull. The microscopic study of the brain revealed the minimal extent of the visible contusion and a slight amount of cerebral edema. This microscopic evidence of cerebral edema cannot be evaluated as a sequela of brain injury, since the extensive parenchymal edema of the various organs was apparently part of the terminal episode and consistent with the severe pulmonary edema. Helpern [4] commented on the unusual limitations of size and extent of the two fissure fractures in the left middle fossa, since fractures in this region, he noted, usually have continuation on the lateral surface of the skull. Of course, there can be no significance of this interesting incidental observation in the consideration of the death, but one can say that the fractures themselves were small and limited and suggested that the trauma to the head was insufficient to cause extension of the fractures along their expected course.

4. Helpern, M.: Personal communication to the author.

The brain presented, then, only minimal changes, and acceptance of these as evidence of death due to injury of the brain necessitates a presumption which would be warranted only in the absence of other possibilities.

Although pheochromocytoma has been studied in detail, and the clinical syndrome of paroxysmal hypertension, paroxysmal tachycardia, vasomotor instability, dyspnea, headache and glycosuria, the histologic changes and the results of bioassay of the epinephrine content of the tumor reported in detail,[5] the limited literature available to me revealed little concerning the details of death resulting from pheochromocytoma. Karsner [2] stated that "death may occur in the attack." Cahill [1] remarked, "Death may occur during an attack from shock, heart failure, coronary disease, cerebral accident, or pulmonary edema"; he further commented, "Not uncommonly, as in several of our cases, the sudden death of a patient with an unrecognized tumor followed by an autopsy discloses the cause." Belt and Powell [3a] noted "great susceptibility to surgical shock with dyspnea, suffocation and pulmonary edema."

In a review of 60 reported cases of pheochromocytoma Belt and Powell [3a] emphasized cardiac hypertrophy and pulmonary edema as the most prominent pathologic alterations. The fatal cases in their series were of two types: those in which the tumor was an incidental finding at necropsy, death being from an entirely unrelated cause, and those in which the tumor appeared to have some relation to the death. In the latter group is evident the tendency of patients with these tumors to die suddenly, for death followed such surgical procedures as hemorrhoidectomy, extraction of a tooth and normal labor. In these cases the chromaffin tumor was found, and the associated pathologic conditions were most frequently pulmonary edema and cardiac hypertrophy of various degrees. Unfortunately, lack of sufficient data precludes deductions as to the relation between cardiac hypertrophy and preexisting hypertension caused by the epinephrine-producing tumor, but it seems reasonable to assume that such is the likely basis for the frequent observation of cardiac hypertrophy in association with tumors of this type. Drake, Hibbard and Hellwig [6] could find no correlation between increased cardiac weight and histologic evidence of increased medullary activity in the absence of hypertension, although in the presence of hypertension such a correlation was noted. Although the cardiac weight was not obtained in this case, there was no gross evidence of cardiac hyper-

5. (*a*) Ewing, J.: Neoplastic Diseases, Philadelphia, W. B. Saunders Company, 1941. (*b*) Christopher, F.: Textbook of Surgery, ibid., 1942, p. 294. (*c*) Cahill.[1] (*d*) Karsner.[2] (*e*) Belt and Powell.[3a] (*f*) Bodansky and Bodansky.[3b] (*g*) Engel and others.[3c]

6. Drake, R. L.; Hibbard, J. S., and Hellwig, C. A.: Arch. Path. **37**:351, 1944.

trophy. The minimal, though extensive, atherosclerosis, involving the mitral valve, the coronary artery and the aorta, cannot be considered of interpretive significance, since it may ordinarily be present in young people.[2]

Of particular interest among the cases reviewed by Belt and Powell[3a] is one reported by Paul: A 54 year old man died "with pressure symptoms in the epigastrium, cyanosis and vomiting." Necropsy revealed a chromaffin tumor, and the author considered this case one of acute epinephrine poisoning. No mention was made of bioassay of the tumor. There was another case of special interest in the same series: A 40 year old man with a clinical history of dizziness, headache and periodic vomiting was clinically considered to be suffering from atropine poisoning. This diagnosis was not substantiated by toxicologic examination, and a chromaffin tumor of the adrenal gland was encountered at necropsy. These cases are suggestive of the possibility of sudden epinephrine-induced crises. It is interesting to note the occurrence of "pressure symptoms in the epigastrium" in Paul's case, and Cahill's[1] observation that "at times epigastric pain is marked," since one of the first symptoms complained of by the patient was "gas on the stomach." It is not known whether the patient had been at stool prior to the attack, but it is known that increased intra-abdominal pressure or massage of the abdomen or of the tumor itself, as in palpation, can precipitate an acute crisis of this type.[7] Such may have been the "trigger mechanism" in this case, though no definite evidence is available.

The role of the pheochromocytoma as the cause of death, then, lacks definitive proof, and in the absence of a bioassay of the tumor it cannot even be stated that the tumor was truly a "functional" one, but the similarity of the terminal episode to those in the reported cases of "acute epinephrinism" with death, the pathologic conditions, the minimal changes in the brain and the suggestive triad of "severe dyspnea, cyanosis and pulmonary edema" with "pressure symptoms in the epigastrium," though in themselves the picture of pulmonary edema, warrant serious consideration when occurring in the presence of a tumor capable of producing such a picture of sudden death.

The third consideration is the possible relation between the injury of the head and the pheochromocytoma. In the cases reported by Belt and Powell,[3a] in which death occurred following ordinarily nonfatal surgical procedures, death followed the precipitating event within a matter of hours, and in clinical cases of acute epinephrine-induced crisis which end fatally after one or two days there is a clinical picture of fulminating hypertension.[3a] A detailed study of the clinical aspects of an acute episode is well presented in the study by Engel, Mencher and Engel[1c] of a patient who was successfully treated by surgical removal of the tumor. In my case there was an interval of four days between the injury of the head and death, which is longer than the intervals in the cases reported by Belt and Powell, and examination of the patient did not yield any evidence suggesting activation of the tumor. The blood pressure, recorded on the night of admission, shortly after the injury, and on the two subsequent days, was completely within normal limits. During his hospitalization the patient had no complaints referable to any system, and if the tumor was directly activated by the injury of the head, such a latent period of four days would be hard to explain.

Of interest is the observation by Elaut,[8] referred to by Drake, Hibbard and Hellwig,[6] that increased activity of the adrenal medulla is definitely associated with injury of the brain. These authors studied the adrenal medulla cytologically, correlating the histologic picture of increased activity with various clinical conditions, and felt that their results confirmed Elaut's observation. They offered no explanation of the possible mechanism of the relationship. The microscopic observation of thrombi undergoing organization in the veins of the adrenal medulla on the affected side offers interesting possibilities in the light of the foregoing observation. The organization of the thrombi indicates that the acute episode did not occur at the time of death but preceded it, and chronologically the adrenal insult could be related to the injury of the head on the basis of the organization of the thrombi. Although the reasons for a relation between an injury of the head or the brain and the adrenal medulla are unknown, one is presented with a possible mechanism here wherein the injury of the brain, though in itself of no great magnitude, resulted in an insult to the adrenal medullary cells with thrombosis of the adrenal veins. The pheochromocytoma, being of medullary tissue, was similarly excited into activity, but the activity remained confined to the tumor until four days later, when, as a result of some unknown "trigger mechanism," the tumor suddenly released a large amount of epinephrine into the blood stream, with an acute fatal episode of hyperepinephrinism.

It is realized that there are many defects in this attractive scheme, that a bioassay would have proved or disproved the "functional" state of the tumor and that a determination of the terminal blood pressure would have been helpful in establishing the status of either shock or fulminating hypertension, but even in the absence of the absolute evidence the clinical picture and the necropsy observations strongly suggest that such a mechanism might have been the cause of

7. Belt and Powell.[3a] Cahill.[1] Christopher.[3b]

8. Elaut, E.: Arch. internat. de méd. exper. **6**:69, 1928; cited by Drake and others.[6]

death, and in evaluating the role of the injury of the brain and the pheochromocytoma combined as a cause of death neither can be completely disregarded, nor can either be completely implicated, on the basis of the necropsy observations.

SUMMARY

In a case of sudden death following injury of the brain necropsy revealed the presence of pheochromocytoma. It is believed that there may have been a relationship between the injury of the brain and the tumor as a combined cause of death.

ADDENDUM

Since submission of this report, my attention has been called to the work of Raab [9] on the toxic effects of epinephrine-like substances. He reported 2 cases of sudden death in young adults in whom the only pathologic change suggesting the cause of death was an excessive myocardial concentration of epinephrine-like substances (epinephrine and related catechols). One death [9c] was marked by gasping respiration and cyanosis; the other occurred during sleep. In both instances some pulmonary edema was observed at necropsy. Neither autopsy revealed an adrenal tumor.

The adrenal medulla produces epinephrine and related catechols which are similar to epinephrine but not identical with it and which are similar in their physiologic effects. These epinephrine-like substances enhance the cardiovascular effects of epinephrine, and the heart has a greater tendency to absorb and accumulate them than any other tissue tested.[9a] The apparent relation between pheochromocytoma and sudden cardiac death on the basis of this chemical physiology is evident, and gives further support to the hypothetic role of pheochromocytoma as the cause of death in the case reported.

9. Raab, W.: (*a*) Exper. Med. & Surg. **1**:188, 1943; (*b*) Arch. Path. **36**:388, 1943; (*c*) **38**:110, 1944.

Notes and News

Appointments, Resignations, Etc.—H. W. Neidhardt, of the department of pathology of the University of Kansas, has been appointed assistant professor of pathology in the University of Texas, at Galveston.

Ivan C. Hall has resigned as professor of bacteriology in the New York University College of Medicine.

C. L. Yuile, assistant professor of pathology in McGill University, Montreal, Canada, has been appointed associate professor of pathology in the University of Rochester, state of New York.

Peyton Rous has become emeritus member of the Rockefeller Institute for Medical Research.

At Wayne University, Detroit, Edgar H. Norris has resigned as dean of the school of medicine and as director of the medical science center; while on leave of absence as professor of pathology at Wayne University, Dr. Norris will be visiting professor of pathology in Washington University, St. Louis

A. W. Webb, formerly assistant professor of pathology in Howard University, Washington, D. C., is now pathologist to Provident Hospital, Chicago.

Mollie A. Geiss has been appointed professor of pathology at the Woman's Medical College of Pennsylvania, to succeed Helen Ingleby, who resigned to become pathologist at the Jewish Hospital, in Philadelphia. Dr. Geiss has been on the faculty of the college since 1923 and associate professor of pathology since 1932.

Awards.—Alexander S. Wiener, serologist to the office of the chief medical examiner, New York, has received the Alvarenga Prize of the College of Physicians, Philadelphia, in recognition of his work on the Rh factors.

Grants in Aid of Cancer Research.—The National Advisory Cancer Council has approved the following grants in aid:

Harvard University, $24,500, for study of the relation of steroid hormones to growth and tumors, under J. H. Means.

Mount Sinai Hospital, New York, $10,775, for clinical studies on gastric cancer, under F. Hollander.

Harvard University, $10,000, for study of the pathology of gastric cancer, peptic ulcer and gastritis, under Shields Warren.

University of Cincinnati, $10,000, for clinical study of gastric cancer, under Leon Schiff.

Northwestern University, $8,500, for research on cancer education, under the supervision of A. C. Ivy.

Haskin's Laboratories, New York, $5,000, for work on the milk factor in mammary cancer of mice, under P. A. Zahl.

Detroit Institute of Cancer Research, Detroit, $6,152, for study of relation of diets to induced cancer in rats, under W. F. Dunning.

New York University, New York, $3,500, for study of Hodgkin's disease, under R. W. Chambers.

University of Minnesota, $1,450, for study of leukemia in mice, under A. Kirschbaum.

Research Fellowships in Public Health.—The United States Public Health Service announces the establishment of National Institute of Health research fellowships. The junior fellowships will be available to holders of master's degrees in the sciences allied to public health. The stipend will be $2,400 per annum. The senior fellowships will be available to holders of doctorate degrees in one of the sciences allied to public health. The stipend will be $3,000 per annum. These fellowships will offer opportunity for study and research at the institute or elsewhere. Address: The Director, National Institute of Health, Bethesda 14, Md.

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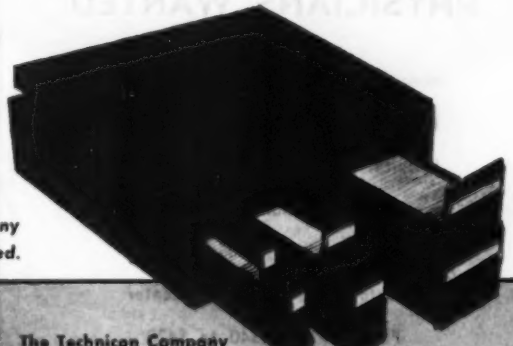

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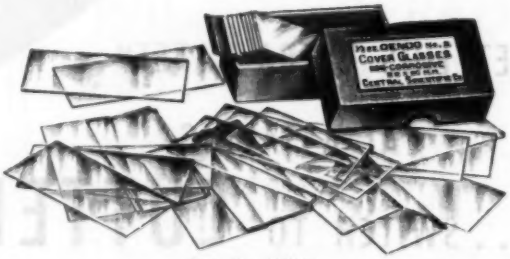

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